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DIAGNOSTIC AND ECOLOGICAL STUDIES OF RICE BACTERIAL

LEAF BLIGHT, CAUSED BY XANTHOMONAS ORYZAE

- Japan -

[Following is a translation of an article by S. Yoshimura in the Japanese-language publication Bulletin of the Hoku-riku Agricultural Experiment Station, No. 5:270176, 1963, pages 28-176.]

TABLE OF CONTENTS

	<u>Page</u>
Chapter I. Introduction	1
Chapter II. History of Nomenclature, Distribution, and Occurrence of the Disease	4
Chapter III. History of Research	23
Chapter IV. Symptoms	26
Chapter V. The Pathophyte of Rice Bacterial Leaf Blight	32
Chapter VI. Bacteriophage of the Pathogen of Rice Bacterial Leaf Blight	51
Chapter VII. Experimental Methods on the Quantitative Test of the Pathogens of Rice Bacterial Leaf Blight	103
Chapter VIII. The Life Cycle of the Pathogen of Rice Bacterial Leaf Blight During the Period of Overwintering	112
Chapter IX. Occurrence and Life Cycle of Rice Bacterial Leaf Blight During the Rice Growing Period	161

<u>TABLE OF CONTENTS (continued)</u>		<u>Page</u>
Chapter	X. Test of Primary Infection	169
Chapter	XI. Test for Secondary Infection	186
Chapter	XII. Relationship Between the Life Cycle of the Pathogen Phage of Rice Bacterial Leaf Blight in Irrigation Water and Disease Occurrence	200
Chapter	XIII. Discussion and Conclusion	260
Chapter	XIV. Synopsis	270
	Bibliography	277
	Photographs	294

CHAPTER I. INTRODUCTION

Rice Bacterial leaf blight is a leaf blight disease commonly called "hagare [leaf blight]," and is the only bacterial disease of the main rice plant diseases in Japan. The record of occurrence of this disease in Japan is old, dating back to 1884. It is a disease that occurs more frequently in the plain, fertile, alluvial rice paddies mainly in the warm southwest region, covering the Kanto district, southwestern Honshu, Shikoku and Kyushu. In foreign countries the disease occurs in almost all rice growing regions of Southeast Asia including Korea, Mainland China, Taiwan, the Philippines, South and North Viet Nam, Laos, Cambodia, Thailand, Malaya, Burma, India, and Colombo [sic]. Generally it is believed to be a rice plant disease of the warm or the semi-tropical regions. However, it has spread from the warm southwestern region north, as will be described later. It has affected the Hokuriku district as well as the Tohoku district.

Although attention has thus been focused on rice bacterial leaf blight as a main rice plant disease throughout Japan, past research on this disease (before 1950) consists of only the difference between varieties, the relationship between a few methods of fertilization and cultivation and the occurrence of the disease, some observations on prevention and eradication by spraying copper germicides. There are no preventive or eradication methods that can be actively recommended for practical use.

The reason for this is that the pathophyte of this disease was found to be complicated and troublesome in isolation, culture and microscopic observation. But more importantly the basic survey and study of the infection cycle, from its overwintering to the primary infection to the secondary infection were inadequate. Because of this, beginning in 1951 the Agricultural Technology Research Center, the Tokai, Kinki and Kyushu Experimental and Research Centers, and colleges have embarked on basic research, with successes, that will be described later, and opened new phases in the study of this disease.

In some of this research the author participated at his former post, at the Kyushu Agricultural Experimental

Station, Ministry of Agriculture and Forestry and engaged in assigned experiments. In parallel with the research, various antibiotic substances have been examined for chemical control. But no definitely effective chemical has been found. Therefore, evasion and reduction of the occurrences of this disease are still dependent on the utilization of resistant varieties, improvement of seed beds, water control in rice paddies, and adjustment of additional fertilization. The lack of control methods for this disease seems to be due to an inadequate grasp of its ecology.

In view of this, the author set test and investigation objectives in fields, and conducted diagnostic study of the symptoms of rice bacterial leaf blight, the form and etiology of the pathophyte, the circumstances of the occurrence of the bacteriophage of this disease and its primary infection and secondary infection, as referred to earlier, and the relationship between the bacteriophage of this disease in irrigation water and its occurrence. Attempts were also made to establish early detection and prevention of its occurrence, and to gain clues to chemical control.

The present study was conducted by the author from 1956 at the Kyushu Agricultural Experimental Station, Ministry of Agriculture and Forestry, at the Hokuriku Agricultural Experimental Station, Ministry of Agriculture and Forestry (hereafter these agencies will be cited without reference to the Ministry of Agriculture and Forestry). In concluding this article the author's heartfelt gratitude is expressed to Professor Doctor Yoshii Hajime of Kyushu University for his cordial guidance and encouragement throughout the study and for reading this article to Mr. Amatatsu Katsumi, Director of the Hokuriku Agricultural Experimental Station, and Doctor Yamazaki Tsutae, Director of Environmental Department of the Station for their thoughtfulness in reading and publishing this article. His gratitude is expressed also to Doctor Tagami Yoshiya, Director of the Kyushu Agricultural Experimental Station and to Doctor Ono Kosaburo, Director of the Hokuriku Agricultural Experimental Station for their guidance and assistance on many occasions since the inception of the study. His thanks are due also to Doctor Mukai Hideo, Director of Pathology and Entomology, Agricultural Research Center for his valuable advice and encouragement, to Doctor Nishizawa Masahiro, Director of Kyushu Agricultural Experimental Station, to Doctor Tamura Ichitaro, Director of the Hokuriku Agricultural Experimental Station, former professor and Doctor Ito Taiichi of Niigata University for his guidance in the study of fundamental bacteriology and its experimental methods, to Doctor Miyamura Sadao, Professor of Niigata University,

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Special notation and gratitude are made for the great aid offered by the staff of the Insect Damage, Agricultural Improvement Sections of Niigata, Toyama, Ishikawa, and Fukui Prefectures, the staff at the Insect Damage Sections of Agricultural Experimental Stations, the staff at Insect Damage Control Centers, and the staff at centers for dissemination of agricultural improvement information.

CHAPTER II. HISTORY OF NOMENCLATURE, DISTRIBUTION, AND OCCURRENCE OF THE DISEASE

Section 1. Nomenclature of the Disease

The Japanese Association of Phytopathology has termed this disease "Bacterial Leaf Blight."⁹⁰ Table 1 lists the local names of this disease as surveyed by the Agricultural Bureau, Ministry of Agriculture in 1931,⁹³ the commonly known names referred to at the Kyushu and Hokuriku Agricultural Experimental Stations from 1955 to 1960, and the names obtained from written answers from agricultural improvement sections and agricultural experimental stations throughout Japan.

Table 1

Common Names of Rice Bacterial Leaf Blight

Common Terms	Areas or Places Used
Shirahagare	Nation-wide
Hagare	Nation-wide
Shirohagare	Northern lake area of Shiga Prefecture where the disease breaks out constantly, Kariya City, Aichi Prefecture, Miyagi Prefecture, Aichi Prefecture
Shiragare	Iwate Prefecture (Nakahirata-mura, Awami-gun), Oita Prefecture, Aichi Prefecture, Saga, Kuji, and Higashi-Ibaraki-gun, Ibaraki Prefecture
Shirogare	Sanpo-gun of Fukui Prefecture, Konan Area and Yamabe Area, Shiga Prefecture, Tsushima Area, Aichi Prefecture, Toyama Prefecture, Oita Prefecture
Jinryoku Hagare	Ehime Prefecture

Sasagare	Kono-cho and part of Osaka Municipality Andon River, Shiga Prefecture Yuki-gun, Ibaraki Prefecture
Shibugare	Entire area of Tokushima Prefecture
Shitabagare	Yabuki area, Hanamaki City, Iwate Prefecture
Shitagare	Southern part of Fukushima Prefecture (Nishishirogawa-gun), Yabuki area, Hanamaki City, Iwate Prefecture, Nishiharu, Atsumi, and Shichiho areas of Aichi Prefecture, Mihara-gun, Awaji-shima, Hyogo Prefecture
Hayagare	Nukata area of Aichi Prefecture
Kanbagare	Narumi area of Aichi Prefecture
Hoshigare	Sarujima-gun, Ibaraki Prefecture
Hohakare	Saga Prefecture
Satagare	Ibaraki-gun and Shinku-gun of Ibaraki Prefecture
Shiraha	Futaba-gun of Fukushima Prefecture, Hyogo Prefecture, Takarameshi area of Aichi Prefecture
Shiraba	Iwate Prefecture
Kareha	Aichi Prefecture
Akaba	Konan and Yamabe areas of Shiga Prefecture
<u>Doroba</u>	<u>Konan and Yamabe areas of Shiga Prefecture</u>
Hazure	Futaba-gun of Fukushima Prefecture
Hagarashi	Yuraku area of Gunma Prefecture
Hamai	Mihara-cho, Mihara-gun of Hyogo Prefecture
Kareagari	Central (Azumi and Tamuraiwa areas) region of Fukushima Prefecture, Awamigun, Yamagata Prefecture, Aichi Prefecture, Toyama Prefecture

Moeagari	Toyokawa area of Aichi Prefecture
Shitabaagari	Shimane Prefecture; Konan, Kamagori, Ichinomiya, Inazawa, Seto, and Shichiho areas of Aichi Prefecture
Shirajakeru	Kosei area of Shiga Prefecture (used by old women)
Kuse	Fukushima Prefecture; Yuraku area of Gunma Prefecture; Yamaguchi Prefecture; Kagawa Prefecture; Hyogo Prefecture; Kyoto Prefecture; Saitama Prefecture; Yamanashi Prefecture; Tochigi Prefecture; Chiba Prefecture; Kanagawa Prefecture; Kochi Prefecture; Ehime Prefecture; Ibaraki Prefecture
Shiraguse	Noriyama City of Chiba Prefecture; Tomiura cho, Toyama-cho, Shirahama-cho, Maruyama-cho, Miyoshimura, Chigura-cho, of Awa-gun of Chiba Prefecture
Haguse	Ehime Prefecture; Aranuma-gun of Ibaraki Prefecture
Gasa	Seino area of Gifu Prefecture; Konan area of Shiga Prefecture (where occurrence is constant); Hyogo Prefecture, Shizuoka Prefecture, Kyoto Prefecture; Ahigara Shimobe and Central area of Kanagawa Prefecture; Vicinity of Hongo-cho, Toyota-gun, Hiroshima Prefecture; Okayama Prefecture; Aichi Prefecture
Kasa	Kanagawa Prefecture; Shizuoka Prefecture Aichi Prefecture
Shirogasa	Kyoto Prefecture; Tsushima area of Aichi Prefecture
Hagasa	Kyoto Prefecture, Hyogo Prefecture, Okayama Prefecture
Gasagasa	Kohoku area of Shiga Prefecture (where occurrence is constant); Shichiho area of Aichi Prefecture
Gara	Tokushima Prefecture; Kagawa Prefecture

Yake	Tochigi Prefecture; Atsumi and Kanie areas of Nagoya of Aichi Prefecture;
Hayake	Tochigi Prefecture; Atsumi and Kanie areas of Nagoya of Aichi Prefecture
Nijiyake	Koryo-cho of Kuzushiro-gun of Nara Prefecture
Hoya	Naganuma-cho, of Iwase-gun of Fukushima Prefecture; Saga Prefecture; Fukushima Prefecture
Shirohoya	Saga Prefecture; Fukuoka Prefecture
Sabai	Amakusa area of Kumamoto Prefecture; Saga Prefecture
Sabiya	Saga Prefecture
Kensaki	Edao-gun of Gifu Prefecture
Kazeatari	Sanbo-cho, Sanbo-gun of Fukui Prefecture
Sasamushi	Daijin, Shimadakata-gun, Izu Peninsula of Shizuoka Prefecture

As shown in Table 1, such names as Kuse, Kasa, and Sabai are general terms to depict other diseases and insect damage. But Gasagasa, Shiraguse, Nijiyake, Kensaki, and Sasamushi literally depict the phases of damage.

Next, the English nomenclature is Bacterial Leaf Blight. It is also called by such other names as bacterial leaf stripe, bacterial leaf streak, leaf withering disease or white spot disease. The first two seem to be the terms designated by Fang et al^{19, 20} for rice streak disease and by Pordesimo¹⁰⁰ for distinguishing it from bacterial blight. In other words, these researchers treat the first two names of bacterial diseases as being different from bacterial leaf blight. Care must be exercised in using names. Incidentally, its Chinese name is Pai-chieh-ku-ping¹⁸. Its German and French names are not known.

Furthermore, the reports on Kresk disease^{102, 106} reported at Bogor, India in 1950, and the rice bacterial disease reported in India show symptoms similar to those of the bacterial leaf blight.⁹ However, the black eroded rice¹³² reported by Cochinal in 1932, Bruzone disease⁵³ of Hungary

in 1949, and un-hulled rice withering bacterial disease since 1953 in Kyushu, Chugoku, Shikoku, Tokai, Kinki, and Fukui Prefecture are different bacterial diseases than this disease judging from symptoms, and pathophyte.

Section 2. Geographical Distribution

As referred to in the introduction, the geographical distribution of rice bacterial leaf blight ranges all over the rice growing zone of Asia, with the exception of Manchuria, Siberia, North Korea. It includes Japan, Republic of Korea,¹²⁸ Mainland China,¹⁸ Taiwan,⁴⁶ the Philippines^{100,101} South and North Vietnam,^{*} Cambodia, Thailand;^{**} Malaya,^{***} Java,¹⁰⁶ Burma,⁹⁸ India,^{9,98} and Ceylon.^{****} Whether or not it occurs in the rice growing zones in California or Northern Italy is not known.

Section 3. History of Occurrence

1. Record of Occurrence in the Early Period

The record of occurrence in Fukuoka Prefecture in Japan in 1884 seems to be the oldest one. Actually there might have been earlier occurrences than this. It occurred thereafter in 1897 in Shimane and Chiba Prefectures and in 1902 in several parts in the warm southwestern region with remarkable damages.^{10,38} This disease tends to occur in places where rice paddies are immersed or inundated. There were other local occurrences. But this became a problem in 1907 in Kyushu, Shikoku, and Chugoku districts. It is reported that the reduction of rice crops by one half due to this disease was not rare in such constant areas of occurrence as Mitsui-gun and Ukiba-gun along the Chikugo

* By correspondence with Dr. Luong Duc Cua, Hanoi City, North Vietnam.

** By conversation with Mr. Piya Giagong, Bangkok, Thailand.

*** By field surveys conducted by Mr. Kawakami Junichiro, Hokuriku Agriculture Experimental Station, Ministry of Agriculture and Forestry.

**** By field survey conducted by Dr. Okamoto Hiroshi, Chugoku Agriculture Experimental Station, Ministry of Agriculture and Forestry.

River in Fukuoka.¹²⁷ In 1923 this disease raged in Ehime Prefecture, causing reduced crops.⁷³ Judging from the foregoing instances, the occurrence of this disease dates back to old times, and it was frequent in the early period of the Meiji era. As reference, Table 2 is an excerpt from the survey of the occurrence of diseases and insect damage in Japan as collected by the Agricultural Bureau, Ministry of Agriculture and Forestry in 1929.

Table 2

Occurrence of Rice Bacterial Leaf Blight in 1926

Prefecture	Area of Distribution	Degree of Damage	Area of Damage	Resume of Occurrence and Damage
Hokkaido				
Tohoku District				
Aomori	Entire area	Small		Noted as
Iwate	Waga and Nishiiwai-gun	Small	2,432.4	The largest damage caused by the disease in recent years
Miyagi	Kurokawa, and Shida-gun	Small	40.0	Occurrence took place for some previous years but with little damage
Akita	Minami-Akita	Small		
Yamagata				
Fukushima				
Kanto District				
Ibaraki	Entire area	Small	1,000.0	Occurrence for some previous years
Tochigi	Kochi, Nasu, Abe	Small	50.0	Occurrence since 1919, frequent among late-maturing varieties
Gunma				
Saitama				
Chiba	Awa-gun	Medium	50.0	Occurrence was noted with the increase in fertilization beginning in 1897
Tokyo	Entire area	Small		

Kanagawa	Entire area	Medium	3,400.0	
Hokuriku District				
Niigata	Mishima-gun	Small		
Toyama				
Ishikawa				
Fukui				
Tosan and Tokai District				
Yamanashi				
Nagano				
Shizuoka	Entire area	Medium	26,175.0	Occurrence from olden times; frequent after flood and storms
Gifu	Entire area	Large	7,804.0	
Aichi	Entire area	Medium	20,000.0	
Mie	Entire area	Large		Frequent among late and middle maturing varieties
Kinki District				
Shiga	Entire area	Medium	11,663.0	
Kyoto	Yamashiro and Tanba-gun	Medium	19,144.0	Tends to increase in recent years
Osaka	Entire area	Small	177.8	
Hyogo	Entire area	Medium	480.0	Particularly frequent in Mihara area
Nara	Ikoma	Small	10.0	Occurrence since about 1902
Chugoku District				
Tottori				
Shimane	Entire area	Large		Occurred in 1917 and 1920 although occurrence was known since 1897

Okayama	Large	11,668.0	Occurrence since olden times, with different damage depending on years
Hiroshima			
Yamaguchi	Entire area		
Shikoku District			
Tokushima	Large	1,544.0	Occurrence since olden times with different degrees and areas of damage depending on years
Kagawa			
Ehime	Small	67,170.0	Occurrence since olden times with different degrees of damage depending on years
Kochi	Small		
Kyushu District			
Fukuoka	Medium		Occurrence since 1884, 1885, widespread in the prefecture in recent years
Saga	Large	50,000.0	Occurrence since olden times; great damage in 1923
Nagasaki	Small	500.0	Different degrees of occurrence and damage, widespread in recent years
Kumamoto	Medium	130.0	
Oita			
	Entire area		
	Oita city, 5-gun		
	10 cho		

Niyazaki	Entire area	Somewhat large	Up to 1921 severe damage was found in northern and western counties, but now it has spread to several places in the prefecture with varied degrees of damage
Kogoshima	Kogoshima-gun, and Chubu-gun	Large	10.0
Okinawa	Occurred on islands used for cultivation		

2. Area of Occurrence

According to the survey conducted by the Epidemic Prevention Section of the Ministry of Agriculture and Forestry, as shown in Table 3, the area affected by the disease in the pre-war period was about 50,000 to 60,000 ha each year. During the Second World War it frequently passed 100,000 ha. In 1945 and thereafter the average area affected was about 100,000 ha. Beginning in 1951, it was increased to 200,000, or twice that of the preceding average, sometimes as large as 300,000-400,000 ha. Recently in terms of area of occurrence, in Japan this disease is important next only to rice plant fever, and spot disease. Table 3 shows the areas of occurrence during the 1937-1960 period.

Table 3

Acreage of Rice Bacterial Leaf Blight in Japan by Year

(1937-1960)

①年次	②作付面積	③発生面積	年次	作付面積	発生面積
	ha	ha		ha	ha
1937	3,069,523	54,688	1949	2,898,750	183,943
1938	3,073,364	40,012	1950	2,901,360	387,066
1939	3,040,906	27,868	1951	2,801,110	220,957
1940	3,029,275	55,002	1952	2,895,830	168,367
1941	3,036,142	118,606	1953	2,889,900	206,512
1942	3,026,026	95,914	1954	2,911,850	310,097
1943	2,991,980	45,104	1955	3,069,970	225,238
1944	2,875,750	40,011	1956	3,084,930	252,732
1945	2,821,070	110,468	1957	3,100,216	410,927
1946	2,742,220	157,266	1958	3,105,000	338,474
1947	2,834,370	115,489	1959	3,131,000	405,553
1948	2,890,090	110,279	1960	3,150,000	347,748

Note: Surveyed by the Plant Epidemic Prevention Section, Development Bureau, Ministry of Agriculture and Forestry

[Legend]: 1) Year; 2) Planted area; 3) Acreage of occurrence.

3. Occurrence in Northern Japan, Particularly in the Hokuriku District^{34,39,152}

For sometime rice bacterial leaf blight was regarded as a main disease in the warm rice growing areas west of the Kanto District and as a negligible one in the Tohoku and Hokuriku Districts. However, even in the latter areas in recent years the occurrence of this disease has rapidly increased. Its occurrence was extended northward from Fukui and Miyagi, with noticeable occurrence in the past several years even as far as Yamagata, Akita, and Iwate prefectures in the Hokuriku and Tohoku Districts. Severe occurrence has been known particularly in the Shonai area of Yamagata Prefecture and in the Hachiro Lake coast of Akita Prefecture. Thus, it is imperative to disregard the idea that this disease occurs only in warm areas.^{34,42,153}

The author conducted field surveys in conjunction with the prefectures of the Hokuriku District in 1958 and 1959 on this disease. Knowledge of the acreage of occurrence and the environment for occurrence gained from these surveys are outlined in the following.

1) Shifts in the acreage of occurrence in the prefectures in the Hokuriku District

Table 4

Acreage of Rice Bacterial Leaf Blight Occurrence in the Hokuriku District Prefectures by Year

(1953-1960)				
Year	Niigata Prefecture	Toyama Prefecture	Ishikawa Prefecture	Fukui Prefecture
	ha	ha	ha	ha
1953		1,520		4,268
1954		1,497	870	2,774
1955		2,810	786	1,960
1956	2,140	7,600	3,286	17,371
1957	3,110	2,960	2,605	24,653
1958	12,176	1,700	4,898	7,544
1959	36,816	6,344	7,344	11,105
1960	14,016	4,652	4,268	14,436

2) Areas of Occurrence in Each Prefecture

The areas of occurrence in the prefectures of the Hokuriku District are shown in the following Tables 5-8.

Table 5

Areas of Occurrence in Niigata Prefecture

Region	Area
Higashi-keijo-gun	Urakawahara-mura: Yasukura River Basin Makimura: Iida River Basin
Naka-keijo-gun	Kakizaki-cho: Haru River, Yoshi River, and Kurokawa River Basins Yoshikawa-cho: Aka, Yoshi and Kuro River Basins Naoetsu Municipality: Yasukawa, Kuwasone, Iida River Basins Takada Municipality: Iida, Nakae, and Tonome River Basins Miwa-mura: Kuwasone River Basin Takashi-mura: Kushichi River Basin Keijo-mura: Yasukura River Basin Iikura-mura: Bessho River Basin
Nishi-keijo-gun	Part of Itouekawa Municipality
Kariba-gun	Hojo-mura: Sabaishi River Basin Kashiwazaki Municipality: Sabaishi, Uno, Mae, and Bessan River Basins Kariba-mura: Bessan River Basin Nishiyama-cho: Bessan River Basin
Naka-uonuma-gun	Kawanishi-cho: Confluence of the Chujo and Sanjo Rivers Toka-cho: Lower course of the Furu, Chujo, Ta, and Kawaharu Rivers
Minami-Uonuma-gun	Muika-cho: Uono River Basin Itsuka-cho: Uono River Basin
Kita-uonuma-gun	Uono River Basin Kochitani Municipality: Iketsu Kawaguchi-cho: Kawaguchi

Mishima-gun	Koshiji-cho: Su River Basin and the lower course of the Shibumi River Nagaoka Municipality: Lower course of the Shichibu Drainage Ditches; Doman River Basin; Lower course of the Kuro- kawa Channel; Lower course of the Mi- shima Channel; Shimazaki River Basin; Joshi and Zendoji River Basins; and Yamakita Channel Basin
Minami-kamabara-gun	Sanjo Municipality: Ozaki and Iguri Mitsuke Municipality: Kono, and Itori Nakanoshima-mura: Mayumi Shimota-mura: Oura Tagami Municipality: Yokoba Shinta
Nishi-kamabara-gun	Naki-cho: Yeroizato Iwamure-mura: Sarugase Shinto-mura: Endo Nishikawa-cho: Hataya Nakano Koya-mura: Nakano Koya Yabiko-mura: Kamiizumi, Ida Ajikata-mura: Okura Kurozaki-mura: Kitaba Tsubame Municipality: Chosho
Naka-kamabara-gun	Niitsu Municipality: Noshiro and Daido River Basins Yokotsuke-mura: Agano River Basin Outside of the Agano River Dikes Kosuto-cho: Outside of the Shinano River Dikes Niitsu Municipality: Outside of the Shinano River Dikes Shirane Municipality: Outside of the Shinano River Dikes Gosen Municipality: Saruwada Muramatsu Municipality: Nagashima
Kita-kamabara-gun	Kamikawa-mura: Migita Mikami-mura: Nodo " " : Ishima Yasuta-mura: Agano River Basin, Windy area Minabara-cho: Windy area Izumigase-mura: Windy area Toyosaka-mura: Around Fukushima Lake Toyoura-mura: " " " Shibata-mura: Kaji River Basin Kurokawa-mura: Tainai River Basin

Iwabuna-gun	Arakawa-cho: Arakawa River Basin Kanbayashi-mura: Ishi and Ara River Basins Murakami Municipality: Ishi, Sanmen, Yamada, River Basins
Niigata Municipality	Niigata Municipality: Kyo and Shinano River Basins, Shindo, Sakai, Nishi River Basin; Okayama, Ishido, Agano River Basin, Omi, Toriyano Lake Channel Area, So River
Sado-gun	Sawada-cho: Kufu River Basin Kanai-mura: Fujitsu River, Shinbo River, Ono River, and Kofu River Basins Hatano-mura: Ogura River Basin Mano-cho: Gonaiden Ryotsu Municipality: Kamo Lake and Ume-tsu River Basin

Table 6

Areas of Occurrence in Toyama Prefecture

Region	Area
Uotsu Municipality	Katagain River Basin Daikoji on the lower course of the Kado River, Sumiyoshi Lower course of the Hayatsuki River
Suberikawa Municipality	Northern part of Suberikawa Municipality, Confluence of Hayatsuki River
Nakashinkawa-gun	Kamiichi River Basin, Joganji River Basin, Shiraiwa River Basin, Funabashi area, Mizubashi area
Toyama Municipality	Higashi-toyama, Hasu-cho, Shirokawabara, Shinjo, Hamagurosaki area (lower course of the Jintsu River), Taromaru, Nezuka, Eguchi, Nishi-toyama
Moi-gun	Kureba-cho, Ida River, Jinmei, Higashi-oita, Fururuguro-mura, Yamada River Basin in Furukawa-mura, Yatsuo-cho area

Kamiaragawa-gun	Kumano River Basin, Kumano Tsugiso, Nakita, Ima-cho, Matsubayashi Jinzu River Basin, Tsugabara, Shima
Shasui-gun	Kosugi-cho
Niiminato Municipality	Sho River, lower course of the Jintsu River, Tsukahara Sakubara Sakudo Area, Shimojo River Basin, Tsubatae
Takaoka Municipality	Tachino in Koyabe River Basin, Higashi- goi, Kuniyoshi, Ishizutsumi Area
Korimi Municipality	Junicho Lake Area
Nishi-tonami-gun	Koyabe River Basin Fukuoka-cho Akamaru, Sakita, Arayashiki, Mikkaichi, Arata, Ishido-cho, Konade, Serikawa, Maniu, Kitakaniya, Sho River Basin in Tode-cho, Tokuichi, Shimoaso, Funatoguchi Channel, Suta, Ichinose, Tonaka-cho Shibue, Hirata, Shimokawa-saki, Shimogokoku, Fukumitsu-cho Takeuchi, Yoshie
Tonami Municipality	Sho River Basin, Senaru, Tobo Higashinaka, Kitatakagi, Nakano-shima, Enami
Higashi-tonami-gun	Fukunocho Takase } Inamic-cho, Toita } Otani River Basin
Shirohata-cho	Yamada River Basin

Table 7

Areas of Occurrence in Ishikawa Prefecture

Region	Area
Enuma-gun	Daiseiji River Basin, Daiseiji-cho Yuminami, South Kawaminami, Southern Coast of Shibayama Lake, Dobashi Tsukimi, Yata-gun, Southern Coast of Imae Lake, Awatsu, Kiba Lake Perimeter

Nomi-gun	Hiratori River Basin
Ishikawa-gun	Taikeiji Channel Basin, Matsutomo-cho, Nonoichi-cho
Kawakita-gun	Kawakita Lake Peremeter, Morimoto-cho, Saita, Imaichi, Yatsuta, Onofu, Tsubata- cho
Kanazawa Municipality	Kawakita Lake Peremeter
Hasaku Municipality	Muratomo Lake Peremeter
Kashima-gun	Kanishi-cho, Shimaya-cho, Kashima-cho, Tazuhama, Nakajima-cho
Hasaku-gun	Takahama-cho, Shiga-cho, Furai-cho,
Hoshi-gun	Kenchi. Monzen-cho, Anamizu, Ukawa
Washima Municipality	Takuta, Yokochi, Koise-cho, lower course of the Machino River
Tamasu Municipality	Ideda, Nonoe, Kumagai, Shoin-cho, Takara- date-cho
Tamasu-gun	Lower Reachers of Ukai River, Kuri-gawa, Lower course of the Shiri River

Table 8

Areas of Occurrence in Fukui Prefecture

Region	Area
Sakai-gun	Kita Lake Peremeter, lower course of the Kutoryu River, Kanatsu-cho, Ozeki, Hyoga, Maruoka-cho, Harue-cho, Kawanishi-cho, Morita-cho
Ashiba-gun	Ashiba River Basin
Yoshida-gun	Fujioka area
Fukui Municipality	Entire area

Katsuyama Municipality	} Kutoryu River Basin
Ono Municipality	
Nioi-gun	Shimizu-cho, Asahi-cho, Hino River Basin
Sabae-cho }	Hino River Basin
Takeo-cho }	
Tsuruga Municipality	
Sanbo-gun	Sanbo-cho Bessho, Mukogasa, Sako, Tomura, Satomuko
Tojiki-gun	Kita River Basin, Otoba, Kiminaka
Obama Municipality	Lower Reaches of Kita River Basin, Minami River Basin
Omeshi-gun	Takahama-cho

Occurrence takes place more in the plain and fertile land along the Sea of Japan in the Hokuriku District as shown in Tables 5-8. All prefectures in the Hokuriku District have low and wet paddies. There are many occurrence areas in these low and wet paddy areas and around lakes scattered all over (Niigata Prefecture: Yoro Lake, Fukushima Lake, Naga Lake; Toyama Prefecture: Junicho Lake; Ishikawa Prefecture: Muratomo Lake, Kawakita Lake, Imae Lake, Kiba Lake, Shibayama Lake; Fukui Prefecture: Kitagata Lake, Five Lakes of Sanbo). This is the characteristic of occurrence in the Hokuriku District. Also in Niigata Prefecture it has been reported that there have been instances of occurrence in the rice paddies in mountainous areas such as Matsushiro-cho of Higashikeijo-gun, Minami and Kita Uonuma-gun's, Netsu-cho of Higashi-kamabara-gun. There are areas of occurrence in Sado-gun, and the inner area of the Noto Peninsula of Ishikawa Prefecture. There is a considerably large area of occurrence in the Ono Basin, Fukui Prefecture.

3) Summary of the Ecological Investigation of the Circumstances for Occurrence

The following is a summary of the pertinent data concerning the environment for occurrence based on the 1958-1959 ecological investigation.

a) Occurrence is frequent in flat, low, and wet paddies.

b) Occurrence is frequent in fourth alluvial formation soil and rare in rice paddies in areas adjacent to sand dunes.

c) Occurrence is frequent in wet paddies and in the rice paddies around lakes.

d) In many cases, paddies where occurrence takes place are flooded by rivers flowing through.

e) The growth and distribution of the host overwintering grass, Leersia oryzoides (Linn.) Sw. of the pathogen of the leaf blight disease have a high density, and naturally affected colonies are found in each prefecture. Host grasses, Zizania latifolia Turcz. ex Trinius, Leersia Japonica Nakino, Phalaris arundinacea Linn., Isachne globosa (Thunb.) O. Ktze also generally grow in the rice paddies of each prefecture and place.

f) Planting sensitive rice varieties like Asahi and others has increased. 39,107

CHAPTER III. HISTORY OF RESEARCH

Rice bacterial leaf blight occurred widely in the warm southwestern region beginning in about 1902, as has been stated. Mr. Nishida, Kyushu Branch of the Agricultural Experimental Station, Ministry of Agriculture and Commerce, made studies of the pathophyte of this disease. In 1907, he reported^{91,92} that this disease was not caused by parasiting bacteria, but that it was a phenomenon caused by the strong acidity of the nutrition absorbed from the soil, because the morning dews formed on the tip of the affected leaf showed acidity. Also such symptoms were only manifested when rice was cultivated in rice paddies with acid solutions and when the solution was changed into a base the same symptoms were not manifested. Therefore, he concluded that the disease was a non-parasitic physiological impediment based on soil acidity. At about the same time, Mr. Maruoka^{74,75} of Shizuoka Prefecture reported that this disease was caused by acid soil. This kind of view was still strongly believed even though Mr. Takaishi of the Fukuoka Prefectural Agricultural Experimental Station published less than two years later the bacterial theory of the disease. This is still believed by the old people in the areas of occurrence in Fukuoka Prefecture. The writer terms this period as the period of the acid theory of impediment by Messers Nishida and Maruyama.

Mr. Takaishi conducted detailed research.^{22,126} At first he discovered that the affected soil had generally stronger acidity than non-affected soil; that soil in heavily affected area showed extreme acidity; the occurrence of this disease was rare in the zones with volcanic ash soil, and in contrast to this the occurrence was frequent in the heavy clay zone with high a substitution capacity. He investigated dew from the affected leaves and found that it had about twice as much acidity as that of non-affected leaves, and that most of it was non-volatile acid containing much organic matter, about three times that of non-affected leaves. From this evidence he considered for a while that the disease was caused by the damage of acidity. But after further research, he discovered paste-like slimy matter on the edge of the leaf of a somewhat yellow tinge at the initial period of disease. Through microscopic inspection he confirmed that this was a mass of bacteria and

reported that this disease was obviously a parasitic disease caused by a kind of bacteria; a characteristic of this disease was that it grew to some extent in the dew drops formed on the leaf edge and penetrated the tissues gradually; that the multiplication of the bacteria in dew drops was related to the ingredients of the dew drops; and that the difference in these ingredients was due to the changes in the physiological condition of rice caused by the soil and fertilization.

His bacterial theory and the foregoing observations are epoch-making accomplishments in the history of this disease and thereafter they were proved by tests. However, Mr. Takaishi only isolated two kinds of bacteria and mould-fungi and did not go as far as their identification. In 1911, Mr. Bokura isolated a kind of yellow bacteria from affected leaves, and termed this pathophyte Bacillus oryzae Hori et Bokura.¹⁰ This bacterium was proved by Mr. Ishiyama, Kyushu Branch of the Ministry of Agriculture and Forestry Agricultural Experimental Station, and he identified that the substantial pathophyte of this disease as Pseudomonas oryzae Uyeda et Ishiyama. He further clarified the nature of the bacteria and its affection cycle, thus completely proving the theory of the pathophyte.⁴⁵

In the meantime, prefectural agricultural experimental stations at Yamaguchi, Shiga, Ehime, Fukuoka, Okayama, Sanga¹⁶, 23, 24, 25, 97, 105, 112, 148 conducted tests on fertilizers, varieties of rice, sources of seedlings, and chemical spraying.

Thus at about the same time, Mr. Soga of the Ministry of Agriculture and Forestry Agricultural Experimental Station, in 1923, and Mr. Shima of the Fukuoka Experimental Station in 1925, conducted a field survey in Kumamoto and Fukuoka Prefectures, respectively, into the history of the occurrence of this disease, its distribution, its damage, the causes of its occurrence, the resistance of different rice varieties, and countermeasures, and collected valuable data. Thereafter Shiga,¹¹³ Ehime,¹⁷ and Kyoto⁶⁷ Experimental Stations conducted several tests on chemicals, fertilizers, and rice varieties. Then in 1927, the Ministry of Agriculture and Forestry entrusted the Aichi Agricultural Experimental Station with the experiments for controlling rice bacterial leaf blight. Mr. Kuwazuka Kikuji, an agricultural engineer, was in charge of the experiment, and they learned much about the symptoms of the disease, the influence of its damage, the ecology of its occurrence, the testing of resistant varieties, and chemical control.^{1-8, 63-66} This experiment lasted until about 1942. The ogyokyu

variety, (shirasenbo x shobee) cross-fertilized on the basis of results gained from inspection tests for disease resistant varieties, is well known. Research was interrupted temporarily during the war. In the post-war period the Agricultural Technology Research Center and the Kyushu Agricultural Experimental Station undertook research once again. Now several prefectural agricultural experimental stations that have in their jurisdictions places of constant occurrence, such as Tokai, Kinki, Chugoku, and Shikoku Districts, Kyushu University and Saga University, have conducted active research. Consequently, many results on the ecology of its occurrence, resistant varieties, the mechanism of occurrence and its pathophyte and phage have been published.

CHAPTER IV. SYMPTOMS

Section 1. Classification of Symptoms¹¹⁸

Since Takaishi,¹²⁶ Ishiyama,^{45,46} Kuwazuka,⁴⁰ and Yoshii⁴⁹ have given descriptions of the symptoms of rice bacterial leaf blight, not many additional words seem to be necessary. But according to investigations by the author, it is necessary to perceive again the process from infection, to its occurrence, and on to its spread thereafter.

In other words, the symptoms of this disease are usually yellow or yellow-white diseased spots forming along the leaf edge. However, the author's investigation has revealed that the symptoms of this disease are considerably different according to its growth period, the amount of infected bacteria, and its environment. The author has classified these symptoms into the four types as shown in Figure 1. The characteristics of these symptoms are listed in Table 9.

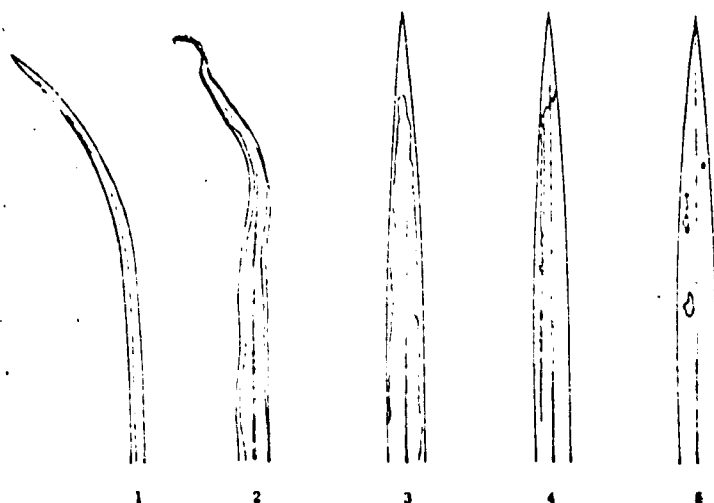


Fig. 1 Symptoms of Bacterial Leaf Blight on Rice

[Legend]: 1) Wilt type (leaf roll); 2) Wilt type (leaf roll); 3) Edge type; 4) Streak type; 5) Spot type.

Table 9

Symptoms of Rice Bacterial Leaf Blight

Item	Symptom	Wilt Type	Edge Type	Streak Type	Spot Type
Shape	Leaf roll-wilting	Water-immersed condition-edge leaf roll Twisting-pale color Pale green color-wilting	Typical wavy	Streaky disease spots appear in the center of leaf blades	Spot types (spots appear in lesions after typhoons)
	Water-immersed condition-edge leaf roll		Streaky disease spots appear in the center of leaf blades	Spot types (spots appear in lesions after typhoons)	
Color	Pale green-bligh	Pale green-bligh	Yellow-white-Ash-white	Light-yellow-brown-ash white-blight	Yellow-Ash white
	In the upper part after development or in extracted leaves	Leaf edges	In leaves except middle leaf ribs and leaf edges	Same as streak type	
Bacterial Ooze	Large	Medium	Small	Rare	
	Leaves whose upper parts have just developed	No particular positions	Same as edge type	Mature leaves after development	
Occurrence Period	Initial to intermediate periods of stump branching	Entire period while in rice paddies	Intermediate period while in rice paddies	After the passing of typhoons	

Of the disease types shown in Table 9, the wilt type seems to be known only slightly, but this is a symptom frequently observed in the initial period of rice growth, and this is a symptom that serves as an important clue to the early discovery of this disease.

1) Wilt Type: Leaves, immediately after development or in the process, roll and wilt, dry up and die. The whole of the leaf or the upper half turn pale white and dies in two or three days. In extreme cases the developed leaves of each branched out culm die. Thus, sometimes its growth is interrupted temporarily, and by "zurikomi (slipping into)" or decaying, the culm is eradicated. (See photographs 2 and 3).

2) Edge Type: This is a typical symptom generally observed during the entire period of growth. This forms white-yellow or ash-white wavy spots on the one or both sides of the leaf edge. (See photograph 1).

3) Streak Type: Streaky white-yellow or ash-white disease spots appear along the leaves, except edges, that is, along veins from leaf tips to the center of leaf blades or in lesions after typhoons. This type is seen much in the host grass, Leersia oryzoides (Linn) Sw.

4) Spot Type: Spot type disease spots of light yellow to ash-white colors appear in the lesions of leaves after the passing of typhoons. They appear more in the lower leaves rather than in the upper developed leaves. This type is seen also in the host grass, Zizania latifolia Trucz. ex Trinius. It is characteristic of this type that it does not spread too much because the siliceous nature of diseases spots is advanced.

As can be seen from the foregoing description, the streak type and the spot type are not seen in the initial period of growth. Also these diseased leaves, in many cases, secrete ooze of the pathophyte of this disease from the lesions on the leaf edge or leaf surface. The amount of ooze of the pathophyte is formed in the largest quantity in the wilt type, and in the edge type, and streak type in that order, and seldom in the spot type. This pathogenic ooze is formed by the pathophytes which multiply in the diseased tissues after they are secreted, coagulated and dried. It is 0.5-2 mm in diameter, and in a granular or paste-like form. Its color is orange-yellow to dirty yellow. In the wilt type several masses of ooze are attached on the inside of the rolled leaf. If sufficient water content from rain drops should be given to these, the leaf

develops outward, and the thick pathophyte-carrying liquid thus formed is dispersed, and plays an important role in the secondary infection. This pathogenic ooze is a sign for discovering the occurrence of this disease in its initial period or for distinguishing healthy leaves from diseased ones. (See photograph 5).

Section 2. Other Diseases Confused as Rice Bacterial Blight and Similar Diseases

The largest impediment for the early discovery of the disease in the diseased culm in its initial stage is the existence of parasitic or non-parasitic diseases or insect damage with very similar symptoms. The author has experienced diseased leaves with similar symptoms which make the distinction difficult. These are enumerated in the following:

Parasitic Diseases

1) Leptosphaerella oryzae (Miyake) Hara:³⁵ This encroaches the tip of the rice leaf and destroys its tissues. When the disease spreads, the diseased leaf is discolored, turns ash white, and dies. It is characteristic of the initial period that diseased spots in streaks are formed from the tip of the leaf on.

2) Fusoma triseptatum Saccardo:³⁵ This forms yellow streaks on the tips of rice leaf blades in July-September. These turn gradually ash white and develop downward. The withered part is dried and contracted in fine weather, and is severed after rainy weather. More frequently, it occurs in rice paddies irrigated with cold water.

3) Virus: Since this disease rolls and droops the leaf, it resembles the wilt type rice bacterial leaf blight. However, in the case of rice bacterial leaf blight the pathogenic ooze which is secreted and forms on the leaf edge, is distinctive.

Non-Parasitic Impediments

- 1) Withering of leaves by wind
- 2) The natural yellowing of lower leaves (aged leaves)

Insect Damage

1) The damaged culm due to Chilo simplex Butler: The damage caused by the rice borer who invades the leaf sheath in the early tillering stage shows distinct yellow streaks from the tip of the leaf on, and the whole culm shows wilting symptoms. Therefore, this seemingly resembles wilted leaves of rice bacterial leaf blight. But this is easily distinguished by the damaged culm which can be easily plucked and the damaged scars are visible.

2) Damaged leaves of Chlorops oryzae Matsumura

3) Encroached and discolored portion by Agromyza oryzae Munakata.

Section 3. Diagnosis of Diseased Leaves Caused by Rice Bacterial Leaf Blight in the Initial Period of Occurrence

1. Diagnosis by Symptoms

As has been stated in the section dealing with the symptoms, there are many wilt types in addition to the edge types in the initial stage of occurrence of this disease, diagnosis must therefore be made with special attention to the existence of the leaf roll in the upper part of the developed leaf and further the pathogenic ooze, which is the manifestation of the disease, must be observed in detail.

2. Simple Diagnostic Method¹¹⁹

As has been stated in Section 2, there are parasitic and non-parasitic diseases and insect damage with symptoms similar to those of rice bacterial leaf blight. The author has devised a simple, effective, and quick method of distinguishing leaves affected with rice bacterial leaf blight from others. This is shown in Figure 2.

As shown in Figure 2, the discolored abnormal rice leaf is collected for diagnosis. This is cut sideways with scissors (or it can be torn into pieces). The tip portion is discarded, and the lower portion is cut to a suitable length (about 5-10 cm), and the remaining portion (the discolored portion) is plunged into the test tube with a suitable amount of water (river water may be used as long as it is transparent). Then the test tube is placed in the test tube rack. When this test tube is observed from the

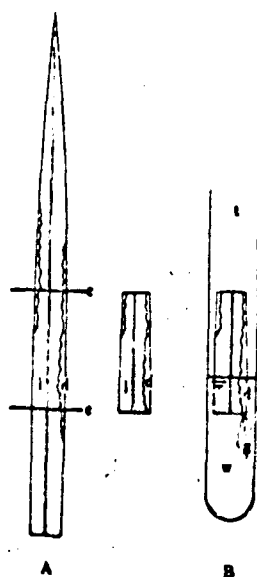


Fig. 2 Showing the Simple Diagnostic Method with Affected Leaf of Rice Bacterial Leaf Blight

[Legend]: A) Affected leaf; B) Test tube; A,1) Leaflet of test sample; c) Cutting; d) Diseased portion; B,t) Test tube; w) Water; g) Secretion of bacteria from affected rice leaf in water.

side, the leaking of white colored pathogeny can be observed in a few minutes (if conditions are suitable, almost instantly), if the leaflet of the test sample is affected with rice bacterial leaf blight. (See photograph 6). Contrarily, if the leaflet is not from an affected leaf, such a phenomenon will not appear however long it may be left in water. The white smoke-like secretion are pathophytes multiplied in tissues, particularly in vascular bundles, which are secreted into water outside the tissues by osmotic pressure. Since bacteria encroachment of rice leaves in Japan is caused by Xanthomonas oryzae alone, the foregoing diagnostic method seems to be tentatively justified. Diagnosis by this method is not valid for dead leaves. Leaves with even slightly green portion must be used.

CHAPTER V. THE PATHOPHYTE OF RICE BACTERIAL LEAF BLIGHT

Section 1. Scientific Nomenclature

The pathophyte of rice bacterial leaf blight was named Pseudomonas oryzae Uyeda et Ishiyama by Ishiyama⁴⁶ in accordance with Nigra classification. However, in accordance with the classification advocated by E.F. Smith, an authority on bacteriology, who respected the priority of naming by Cohn (1897) who classified polar flagella in the category of Bacterium, it was claimed that Nigula's Pseudomonas should be properly changed to Bacterium. Thus, the pathophyte of rice bacterial leaf blight was changed to Bacterium oryzae (Uyeda et Ishiyama) Nakata (1927). Then the classification method of bacteria was discussed thereafter. Consequently, it was widely approved by phytopathologists that non-sporing polar flagella Gram negative rods that form yellow colonies as rice bacterial leaf blight be placed in the family of Xanthomonas as established by Dowson (1939).¹⁴ At present, Xanthomonas oryzae (Uyeda et Ishiyama) Dowson^{11,15} is used generally as the scientific nomenclature. In addition to the aforementioned, it has, in accordance with the classification of bacteria, a different name, Phytomonas oryzae (Uyeda et Ishiyama) Magrou (1937).

Section 2. Isolation

This pathophyte can be easily isolated by the usual method of bacterial isolation. A simple and sure method is to process at first the freshly affected leaf before it shows leaf roll and wilting. In this case an affected leaf which has yellow diseased spots that run lengthwise along the leaf vein in the center of the leaf blade, not along leaf edge, must be selected for isolation. It is cut sideways into a 2-3 cm piece and dipped in 96-98% mixture of Ethanol for 30 seconds, and then in 1,000 time mercury bichloride solution for one minute for strong surface sterilization. Then, this is rinsed with sterilized water. The tissue piece is cut with flame-sterilized scissors to the lengthwise diseased spot. Then the diseased spot is torn off lengthwise along the leaf vein by sterilized tweezers and placed on the plate of a Potato semi-synthetic agar medium (hereafter referred to as semi-synthesis). This is maintained at 28°C. On the fourth day yellow, wet, and

glossy colonies of pathophytes are formed around the tissue piece, and then this is transferred to a slope medium of semi-synthesis. After the growth of cultured bacteria on the slope, single colony isolation by dilution cultivation is made. This completes almost pure isolation.

Table 10

Composition of Potato Semi-synthetic Agar Medium

Potato	200.00	gr
Ca (NO ₃) ₂	0.5	
Na ₂ HPO ₄ · 12H ₂ O	2.0	
Cane sugar	15.0	
Pepton	5.0	
Water	1000.0	ml
Agar	25.0	gr
	pH 7.0	

Section 3. Morphology

Ishiya⁴⁵ already has detailed records of observation on the pathophyte of rice bacterial leaf blight, Xanthomonas oryzae. However, the author desires to give a description of the outward shape of the pathophyte through the electron microscope and through the optical microscope.

1. Shape¹⁶⁷

1) Optical Microscope Observation

a) Cultivated bacteria: Bacteria from H5806, H5840, H5839, H5831, H5918, H5915, H5803, H5814, and H5826, in the possession of the Hokuriku Agricultural Experimental Station, from Shijo and Benikonaya were cultivated in the semi-synthetic agar medium at 28°C on the slope for 24 hours. Test samples for the slide were made from the colonies of thus cultivated bacteria. These were stained either with Ziehl carbolio acid Fuchsine or with 5% carbolio acid Gentian Violet. Observation by optical microscope showed that the majority of the bacteria were rod shaped, but sometimes vibrio shape or were irregular globular bacteria as shown in Figure 3. The bacterial cells were usually dispersed, but sometimes two or three cells seemed to grow together.

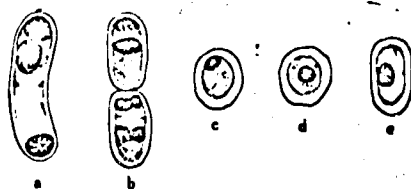


Fig. 3 The Shape of Xanthomonas oryzae

[Legend]: a) Vibrio command shape bacterium;
b) Fission fungus, 2 grow together; c-d)
Globular and irregular shape bacteria; e)
Regular rod-shape bacterium.

b) Bacteria multiplied in disease tissues (hereafter referred to as diseased leaf pathophyte):

Fresh affected leaves are collected and given surface sterilization. Twenty leaves are made into bundles with thread. Their lower part is cut with a razor, and the cut edges are inserted in large test tubes (with about 20 ml of sterilized water). Then in several minutes, the bacteria which multiplied in the vessels are secreted into the sterilized water. (See photograph 6).

When bacteria are thus allowed to secrete for about three hours, about 10^{7-8} /ml of the suspension of the diseased leaf pathophyte is gained. After this was sufficiently shaken, it was stained on the slide as the test bacteria liquid and the test pieces stained by the aforementioned method were observed. Its result showed that the bacterial cells of the diseased leaf pathophyte were in many cases grouped in a mass or bundle. The shape of individual bacterial cells were short rod or oval shape globular bacteria. And it was observed that there were few typical rod shape and Vibrio shape as observed in the cultivated bacteria. It was noteworthy in this experiment, that the dispersion of the pathophyte was extremely bad and there were many short rod shape bacteria. (See photographs 11-12).

2) Electron Microscope Observation

a) Cultivated bacteria: Akashi TRS-50 type electron microscope (accelerated electric pressure 50 KV) was used. Bacteria from Shinjo, Benikonaya, H5839, H5925, H5913, and H5921 were cultivated on the slope in the

semi-synthetic agar medium at 23°C for 24 hours. A very small amount of bacteria from the cultivated bacterial colonies was gathered with a sterilized glass needle, and this was mounted in sterilized water on the mesh or on a osmotic pressure buffer solution of germicide Bernard.¹⁷⁴ (See photograph 10). Then it was dried naturally in the room Berger. A part of this was inspected transparently, and another was tested microscopically after being made into a sample with chrome shadow casting.

The results in both cases were rod and short rod shape bacteria. And it was discovered that *Vibrio* shape bacteria observable by an optical microscope were stained bending cells just before division. (See photograph 13). It was determined that the rod shape was the principal shape of the bacteria. Furthermore in this experiment many granular tubercles on the surface of the bacterial cell and the capsul-like membrane entity on the mature body were observed.

b) Diseased leaf pathogen: The affected leaf with the streak type diseased spot was cut into a pen-point shape with the lengthwise diseased spot as the tip. Its tip was dipped in the sterilized water on the mesh or in an osmotic pressure buffer solution such as sterilized Bernard. The bacteria in the diseased tissue were secreted, then mounted. (See photograph 10) Or, the suspension of the pathogen made in a method similar to the one described in the preceding section was mesh mounted for natural drying in the room. Then this was either chrome shadow cast or inspected transparently.

The result was that there were many bacteria chained and grouped in masses and bundles as in the case of optical microscopic observation. Also the shapes of individual cells showed more short rod or globular shape, than cultivated bacteria.

2. Size

1) Optical Microscope Measurements

a) Cultivated bacteria: Strains from H5806, H5840, H5826, H5808, H5921, H5814, H5831, H5839, and H5915 in the possession of the Hokuriku Agricultural Experimental Station were cultivated on the slope by the semi-synthetic agar medium and the meat extract agar medium at 23°C for 48 hours. Bacteria taken from the grown colonies were mounted on the slide as painted test samples. These were stained with 5% carbolic acid Gentian Violet, and their sizes were

measured with the micrometer attached to optical microscope.

The shortest diameters and longest diameters of the 100 bacteria cells of each strain were measured. The results are as shown in Tables 11 and 12.

b) Diseased leaf bacteria: The results of the measurement of the diseased leaf bacteria by a similar method as described in 1. (affected rice plant varieties: Echigo nebari, Yoneyama, and Kinnanpu) are shown in Table 13.

c) Observation results: As shown in Table 11, the measurements of the cultivated bacteria with the semi-synthetic agar medium are 0.492 , the mean shortest diameter, and 1.48μ , the mean longest diameter. The fluctuation ranges are $0.3-0.6 \mu$ for the shortest diameter, and $0.9-2.2 \mu$ for the longest diameter. The measurements of the cultivated bacteria with the meat extract agar medium are 0.44μ , the mean shortest diameter, 1.52μ , the mean longest diameter, and their fluctuation ranges are $0.4-0.5 \mu$ for the shortest to $0.9-1.9 \mu$ for the longest diameter. In contrast to this, the measurements of the diseased leaf bacteria are, as shown in Table 13, 0.41μ , the mean shortest diameter, 0.796μ , the mean longest diameter, and the fluctuation ranges are $0.3-0.5 \mu$ for the shortest to $0.5-0.7 \mu$ for the longest diameter. It is obvious that the latter have smaller measurements compared as with the cultivated bacteria.

Table 11

Measurements of the Pathogens of Rice Bacterial Leaf Blight (Potato Semi-synthetic Agar Medium)

Strain No.	Shortest Diameter		Longest Diameter	
	Mean Value	Range of Measured Values	Mean Value	Range of Measured Values
H5806	0.48μ	$0.4-0.5 \mu$	1.64μ	$1.3-2.2 \mu$
H5840	0.47	$0.4-0.5$	1.57	$1.2-2.1$
H5825	0.445	$0.3-0.45$	1.27	$0.9-1.7$
H5808	0.53	$0.5-0.6$	1.55	$1.3-1.8$
H5921	0.55	$0.5-0.6$	1.62	$1.1-2.1$
H5814	0.50	$0.5-0.6$	1.33	$1.0-1.9$
H5831	0.49	$0.4-0.5$	1.26	$1.1-1.8$
H5839	0.48	$0.4-0.5$	1.44	$1.1-1.7$
H5915	0.48	$0.4-0.5$	1.61	$1.2-2.0$
Mean	0.42	$0.3-0.6$	1.48	$0.9-2.2$

Table 12

Measurements of the Pathogens of Rice Bacterial
Leaf Blight (Meat Extract Agar Medium)

Strain No.	Shortest Diameter		Longest Diameter	
	Mean Value	Range of Measured Values	Mean Value	Range of Measured Values
H5806	0.44 μ	0.4-0.5 μ	1.58 μ	1.2-1.8 μ
H5840	0.44	0.4-0.5	1.57	1.1-1.7
H5826	0.41	0.4-0.5	1.43	0.9-1.7
H5808	0.46	0.4-0.5	1.57	1.2-1.9
H5921	0.45	0.4-0.5	1.59	1.2-1.9
H5814	0.43	0.4-0.5	1.59	1.1-1.9
H5831	0.42	0.4-0.5	1.42	1.0-1.8
H5839	0.46	0.4-0.4	1.45	1.0-1.9
H5915	0.45	0.4-0.4	1.48	1.1-1.8
Mean	0.44	0.4-0.5	1.52	0.9-1.9

Table 13

Measurements of the Pathogens of Rice Bacterial
Leaf Blight (Bacteria Multiplied in Tissues)

Rice Variety of Affected Leaf	Shortest Diameter		Longest Diameter	
	Mean Value	Range of Measured Values	Mean Value	Range of Measured Values
Yoneyama	0.40 μ	0.3-0.5 μ	0.72 μ	0.5-1.1 μ
Echigonebari	0.42	0.3-0.5	0.88	0.5-1.7
Ninnanbu	0.42	0.3-0.5	0.79	0.5-1.2
Mean	0.41	0.3-0.5	0.796	0.5-1.7

2) Electron Microscope Measurements

a) Cultivated Bacteria: The bacteria from the strains of Shinjo, Benikonaya, H5839, H5925, H5913 and H5921 in the possession of the Hokuriku Agricultural Experimental Station were cultivated with the semi-synthetic slope agar medium at 23°C for 24 hours. The test samples from these were mounted on mesh, by the same method as described in 1, chrome shadow-cast and photographed at 4,000 magnifications. The measurements were made from 100 arbitrarily selected pieces and 2,000 magnification prints from the photographic negatives were enlarged five times. The results are shown in Table 14.

Table 14

Measurements of the Pathogens of Rice Bacterial Leaf Blight

Strain No.	Lyso-type	Shortest Diameter		Longest Diameter	
		Mean Value	Range of Measured Values	Mean Value	Range of Measured Values
Shinjo	A	0.65 ^μ	0.56-0.86 ^μ	1.82 ^μ	1.43-2.62 ^μ
Benikonaya	B	0.61	0.50-0.68	1.53	1.18-1.87
H5839	C	0.65	0.56-0.75	1.73	1.43-2.25
H5925	D	0.65	0.57-0.75	1.85	1.30-2.25
H5913	E	0.66	0.56-0.75	1.80	1.80-2.18
H5921	A	0.65	0.56-0.75	1.69	1.25-1.86
Mean		0.65	0.55-0.75	1.74	1.35-2.17

Note: Cultivated by the potato semi-synthetic agar medium at 23°C for 24 hours.

b) Diseased Leaf Bacteria: The diseased leaf bacteria (affected varieties: Echigo early maturing, Norin No. 29, Kinnanpu) were measured as described in 2)1. The measurements were made by the same method as in the preceding section.

The results are shown in Table 15.

Table 15

Measurements of the pathogens of Rice Bacterial
Leaf Blight (Bacteria Multiplied in Tissues)

Rice Variety of Affected Leaves	Shortest Diameter		Longest Diameter	
	Mean Value	Range of Measured Values	Mean Value	Range of Measured Values
Echigo early maturing	0.49 μ	0.45-0.62 μ	0.89 μ	0.55-1.7 μ
Narai No. 29	0.50	0.43-0.57	0.77	0.59-1.5
Kinsanpu	0.49	0.47-0.60	0.80	0.59-1.6
Mean	0.49	0.45-0.60	0.82	0.65-1.40

c) Observation Results: As shown in Table 14, the measurements of the cultivated bacteria with an electron microscope are 0.65 μ , the mean shortest diameter, 1.74 μ , the mean longest diameter, 0.55-0.75 μ , and 1.35-2.17 μ , their respective range of fluctuation. The measurements of the diseased leaf bacteria are 0.49 μ , the mean shortest diameter and 0.82 μ , the mean longest diameter; their respective range of fluctuation is 0.45-0.60 μ , the shortest diameter and 0.65-1.40 μ , the longest diameter. That the measurements are smaller than those of the cultivated bacteria agrees with optic microscopic observation. The measured values obtained from the electron microscope seem to be somewhat larger than those for the optical microscope for both the shortest and longest diameters.

3. Flagella

The result of flagella staining of the pathogen of this disease by the Lembach and Sous method⁷³ revealed that the pathogen has a single polar flagellum, and this was observed to be a typical polar monotricha. The observation made with an electron microscope showed the same result. The pathogen with two single polar flagella as described by Ishiyama⁴⁵ was not observed. According to measurements made by enlarged photographs using an electron microscope, the width of the flagellum of this pathogen is about 30 microns, its largest length is 8.75 microns, and it zigzags 5-7 times. According to the classification of flagella by E. Lofson,⁷² it belongs to Polar Monotrichous, and the wavelength/amplitude ratio is 10:1, and of the normal type.

4. Capsule-like Membrane

The cultivated bacteria of this pathogen at 28°C for 24 hours were membrane-stained by the Hiss¹³ and the Novelli method,⁹⁵ investigated with an optical microscope, and observed at the same time with an electron microscope. The results were stain positive according to both the Hiss method and the Novelli method. According to the former method the presence of the capsule-like membrane was positively proved. Also by the electron microscope, the non-structural capsule-like membrane as the complete gelly type entity was observed. (See photographs, 8, 9, 15).

5. Gram Staining

The Gram's method of staining was made on the colonies of strains of H5801, H5849, H5839, H5913, H5925 and H5921 in the possession of the Hokuriku Agricultural Experimental Station, slope cultivated in the semi-synthetic agar medium at 28°C for 24 hours, and the bacteria produced from affected leaves of Kinnanpu, Echigo early maturing, and Morin No. 29 were stained by the Gram's method in the normal way.¹³ All showed negative results.

6. Motility

Strains from Shinjo, Benikonaya, H5839, H5913, H5925 and H5921 in the possession of the Hokuriku Agricultural Experimental Station were cultivated in a semi-synthetic agar medium at 28°C for about 36 hours. A platinum loop-full of the bacteria thus produced was taken to the covered glass, and placed inversely on the glass for a microscopic inspection of their motility. It was observed that the bacteria made rapid and active forward moving motions. However, when the period of slope cultivation is over five days, the bacteria becomes inactive and in many cases shows a movement similar to the Brown movement.

7. Summary

The results of the foregoing experiments are summarized in Table 16.

A comparison of Table 16 with the results reported by Ishiyama⁴⁵ reveals that while Ishiyama reported the sizes of the bacteria in the host to be 0.8-1.0 x 0.7 microns, the measured values of the bacteria by the author using an electron microscope were 0.49 x 0.82 microns. While Ishiyama observed two single polar flagella, the author observed only a single polar flagella in all cases. The presence of

the capsule-like membrane was clarified, and the granular protuberances on the surface of bacteria was recognized.

Table 16
Morphology of Xanthomonas oryzae

Properties	Cultivated Bacteria	Diseased Leaf Bacteria (Bacteria multiplied in Tissues)
Shape	Rod-short rod	Short-rod-globular
Bacterial axis	Linear	Same
Bacterial edges	Round	Same
Bacterial side	Parallel-round	Same
Arrangement	Individually diffused	In masses, and hard to diffused
Bacterial cell surface	Granular bumps	
Capsule-like membrane	Hiss-stain positive Novelli stain positive Jelly or Gel shape Capsule-like membrane as observed with electron microscope	Unknown
Staining	Easily stained with basic colors	Same
Motility	Active	Inactive
Flagella	Single polar	Same
Size	0.492 x 1.48 microns 0.440 x 1.52 microns (by optical microscope) 0.65 x 1.74 microns (by electron micro- scope)	0.41 x 0.796 microns (by optical micro- scope) 0.49 x 0.82 microns (by electron micro- scope)
Gram staining	Negative	Same
Others	Flagella wavelength/ amplitude ratio 10:1 (normal type)	

Section 4. Host Range

1. Results of Past Experiments

In order to determine the host range of the pathogen of rice bacterial leaf blight, Kuwazuka⁶³ attempted

inoculation of several grasses, including barnyard grass, but without occurrence of the disease. Tomiyama, et al¹³³ investigated the presence of the disease by needle inoculation in Indian corns, corns, millet, Setaria viridis Beauv., Digitaria ciliaris Pers., Oshiba (sic), barnyard grass, and Zizania latifolia Turcz. The presence was observed only in Zizania latifolia Turcz. Goto, et al¹²⁷ experimented with similar needle inoculations in 41 kinds of grass and reported the presence of the disease in Zizania latifolia Turcz., Phalaris arundinacea Linn., Leersia oryzoides (Linn.) Sw. var. Japonica (Retz) Honda, Phragmites communis Trin. and Isachne globosa (Thunb.) O. Kuntze. Natural occurrence was present in Leersia oryzoides (Linn) Sw. var. Japonica and Zizania latifolia Turcz.

2. Pathogenicity of Leersia oryzoides (Linn.) Sw. and Setaria viridis Beauv.

Pursuing the idea that there might be new host plants of this pathogen other than the ones proven by past tests, the author conducted the following inoculation tests on 14 grasses growing nearby in the rice paddies of the Hokuriku Agricultural Experimental Station.

1) Test Method

The affected leaves of the rice variety, Sanin No.52 were collected. The diseased sections were cut with scissors and ground in the mortar. A proper amount of water was added. Then they were filtered through cheese cloth. Eight strains, as shown in Table 16, were selected from the bacteria thus ground from the diseased leaves (bacterial density 107/ml) and from the preserved bacteria of the Hokuriku Agricultural Experimental Station, each of the test grasses was inoculated (Needle bundle 5).

2) Test Results

The results of the pathogenicity testing of grasses using the diseased leaf bacteria are as shown in Table 17.

As shown in Tables 18 and 19, the 1959 inoculation tests revealed that in addition to the known host plants, Leersia oryzoides (Linn.) Sw, Paspalum Thunbergii Kunth, Digitaria ciliaris Pers., Setaria viridis Beauv showed a slight degree of pathogenicity. Therefore, inoculation tests were made again in 1960. For inoculation the 85905 strain with comparatively strong pathogenicity among the bacteria preserved by the Hokuriku Agricultural Experimental Station was selected. The test results are shown in Table 20.

Table 17

Pathogenicity of Grass on Rice Bacterial Leaf Blight
Pathogen (Diseased Leaf Bacteria, Inoculation)

Name of Grass	Number of Leaves Inoculated	Presence of Disease		Remarks
		Presence	Diseased Leaves	
<i>Leersia oryzoides</i> (Linn.)	76	+	68	Inoculated on 1 September
Ashitaki (sic)	42	+		Presence of disease was investigated on 1 October
<i>Zizania latifolia</i> Turcz.	113	+		
<i>Phalaris arundinacea</i> Linn	22	+	5	
<i>Isachne globosa</i> (Thumb)	38	+	3	*
<i>Panicum Crusgallii</i> L. var. <i>echinata</i> Mukino	71	±	2	The vicinity of the pin- holes made by the inocula- tion needle turned yellow. This was taken as indi- cating a slight parasitic nature of the pathogen. Thus + was used for this, with the numerical values.
<i>Paspalum Thunbergii</i> Kunth	45	±	2	
<i>Digitaria ciliaris</i> Pers.	51	±	5	
<i>Setaria viridis</i> Beauv.	53	±	2	
<i>Rottboellia compressa</i> L. f. <i>japonica</i> Hack	76	±	1	
<i>Miscanthus sinensis</i> Anderss	34	±	2	

Table 16

Pathogenicity of Grasses on Rice Bacterial
' f Blight Pathogen

Grass	Strain No.	Number of Inoculation	Degree of Occurrence*				
			###	##	++	+	-
<i>Leersia oryzoides</i> (Linn.)	H5801	39	26	13			
	H5823	43	43				
	Benikonaya	49	20	22	4	3	
	Shinjo	9	3	6			
	H5840	30	7	6	13	4	
<i>Zizania latifolia</i> Turcz	H5849	34	21	5	7	1	
	H5836	22	10	7	3	2	
	H5839	11	1	5	4		
	H5801	53			1	12	39
	H5823	43	2	4	3	11	25
<i>Ashikaki (sic)</i>	Benikonaya	21		1	3	5	8
	Shinjo	23		3	4	12	4
	H5840	24		1	3	3	12
	H5849	25			2	6	9
	H5836	22			1	7	10
<i>Ashikaki (sic)</i>	H5839	40			1	19	5
	H5801	12			1	7	2
	H5823	13			2	6	1
	Benikonaya	13			1	3	6
	Shinjo	9		2	1	3	1
<i>Ashikaki (sic)</i>	H5840	16			1	7	1
	H5849	11				4	3
	H5836	20				6	8
	H5839	11				2	7
	H5839	11				2	7

Phalaris arundinacea (Linn.)	H5801	8			3	1	4
	H5823	7			5		2
	Benikonaya	11			6	3	2
	Shinjo	9			6	2	1
	H5840	8			4		4
Isachne Globosa (Thumb)	H5849	4			2		2
	H5826	6				2	4
	H5839	8			3	1	4
	H5801	28			11	8	9
	H5823	25			3	3	19
Paspalum Thunbergii, Kunth.	Benikonaya	30			16	8	6
	Shinjo				4	1	2
	H5840	12			4	3	5
	H5849	16			6	8	2
	H5836	19			4	6	9
Panicum crusgalli L. var. echinata Makino	H5839	15			1	3	11
	H5801	17			2	3	12
	H5823	20			7	2	11
	Benikonaya	8			5	3	
	Shinjo	41			14	10	17
Panicum crusgalli L. var. echinata Makino	H5840	17			8	2	7
	H5849	6				1	5
	H5836	33				1	32
	H5839	32			3	1	23
	H5801	19		1	7	6	4
Panicum crusgalli L. var. echinata Makino	H5823	41			10	18	13
	Benikonaya	32			2	13	17
	Shinjo	17			3	8	6
	H5840	9			1	3	5
	H5849	13			2	5	6
Panicum crusgalli L. var. echinata Makino	H5836	16				3	13
	H5839	22					22

- * Criteria for the degree of occurrence
 - Pin holes are points of inoculation.
 - ± The vicinity of the points of inoculation turn yellow-brown, but it is difficult to recognize this as a diseased spot.
 - + The area of the diseased spot at the point of inoculation is about 4 mm².
 - + " 9 mm²
 - +++ " 15-25 mm²
 - +++ " 35-49 mm²
 - ++++ " over 49 mm²
- ** The vicinities of the points of inoculation on Paspalum Thunbergii Kunth and Panicum crusgalli L. var. echinta Makino changed color and appeared to be diseased spots. However, the second isolation of bacteria was a failure.

Table 19

Comparison of Pathogenicity of Grass on the Rice Bacterial
Leaf Blight Pathogen (Frequency of Occurrence*)

Inoculated Strain No.	Leersia (Linn) Sw.	Zizania Latifolia Turcz.	Ashikaki (sic)	Phalaris arundinacea Linn.	Isachne (Thunb)	Paspalum Thunbergii Kunth	Panicum crus-galli	Mean
H5801	53.4	6.3	5.4	1.9	2.0	0.6	3.4	10.4
H5823	60.0	9.7	7.7	3.6	0.6	1.8	1.2	12.1
Benikonaya	44.8	9.1	6.2	2.7	2.7	3.1	0.3	9.8
Shinjo	46.7	13.4	19.3	3.3	2.9	1.7	0.9	12.6
H5840	32.0	7.9	7.8	2.5	1.7	2.4	0.6	7.8
H5849	47.4	2.0	1.8	2.5	1.9	0	0.8	8.1
H5836	43.7	2.0	1.5	0	1.1	0	0	6.9
H5839	34.6	2.6	0.9	1.9	0.3	0.5	0	5.8
Mean	45.3	6.6	6.3	2.3	1.7	1.3	0.9	

*The frequency of occurrence of the disease was calculated by following formula based on the criteria for the degree of occurrence.

$$\text{Degree of Occurrence} = \frac{(40 \sum \text{###}) + (40 \sum \text{##}) + (20 \sum \text{#}) + (10 \sum \text{+}) + (5 \sum \text{+}) + (9 \sum \text{+})}{\text{Gross number investigated}}$$

Table 20

Pathogenicity of Grasses on the Pathogen of Rice Bacterial Leaf
Blight (H5905 inoculated with strong pathogenicity)

Grass	Number of inoculated Leaves	Affected Leaves	Rate of Occurrence	Remarks
<i>Panicum crusgalli</i> L. var. <i>echinata</i> Makino	117	± (14)	(13.5)%	Needle inoculation: 22 July
<i>Paspalum thunbergii</i> Kunth.	103	± (7)	(6.8)	Investigation of occurrence: 12-17 August.
<i>Digitaria ciliaris</i> pers.	24	- 0	0	
<i>Rottboellia compressa</i> L.F. Var. <i>japonica</i> Hack	55	± (5)	(10.0)	
<i>Isachne globosa</i> (Thunb)	42	42	100.0	Investigation of Occurrence: 6 Oct.
<i>Zizania latifolia</i> Turcz	48	48	100.0	
<i>Leersia oryzoides</i> (Linn) Sw.	40	40	100.0	
<i>Setaria viridis</i> Beauv.	81 156	11* 8 (23)	13.6 5.1**	* Successfully isolat- ed. ** Inoculation on 29 August

Note: ± stands for slight yellowing or reddish brown in the vicinity of the needle hole, and the figures are shown in ().

As shown in Table 20, Leersia oryzoids (Linn.) Sw. showed clear-cut pathogenicity. Setaria viridis Beauv also showed occurrence, differing from the known results. Therefore, bacteria were isolated from the diseased spots, and the isolated bacteria were inoculated on the rice (variety: Toishi, sowed on 12 August) transplanted and raised in a pot on 20 September. Tests were made to determine whether the isolates were the pathogens of rice bacterial leaf blight. At the same time, bacterial reactions and lyso-types were determined by phage affinity. The results are shown in Table 21.

Table 21

Pathogenicity of Isolate from Setaria viridis Beauv. on Rice Plant

Number of isolated strains	Pathogenicity of Rice Plant	Lyso-types of isolates	Remarks
6	+	6A type strains	The inoculated bacteria on <u>Setaria viridis</u> is H5905 (Type A).

Note: The rice variety used for inoculation was Kinnanpu.

The symptoms in Setaria viridis Beauv. caused by the inoculation are manifested lengthwise beginning from the inoculated part along the leaf vein. The spots are light-yellow to white yellow. The parts bordering the healthy parts are of somewhat yellow, manifesting symptoms peculiar to the vessel disease.

3) Observation of results

As has been stated in 1, it was concluded from past results that Digitaria ciliaris Pers., Setaria viridis Beauv., Rottcellia compressa L.F. var. Japonica Hack, and Miscanthus sinensis Anderss. had no parasiticity. However, as shown in Table 20, occurrence was observed in Setaria viridis Beauv when inoculated, even though the rates were low at 5.1-13.6%. The bacteria were isolated, and the parasiticity of this grass was identified. However, occurrence was not observable at all with spray inoculation tests conducted separately. From this it is speculated that occurrence in natural conditions is very rare. In

this experiment, pathogenicity seemed to be manifested in barnyard grass, Miscanthus sinensis Anderss. But the re-isolation and identification of the bacteria have not been made.

From the foregoing it was found that Leersia oryzoides (Linn.) as its variant, Sayanakagusa (sic) naturally grown in the Hokuriku District, is a very susceptible host plant.

CHAPTER VI. BACTERIOPHAGE OF THE PATHOGEN OF RICE BACTERIAL LEAF BLIGHT

Section 1. Isolation and Identification of the New Bacteriophage, OP₂¹⁶⁰

1. Testing the Bacteriophage in the Hokuriku District

The bacteriophage (hereafter simply referred to as phage) of the pathogen of rice bacterial leaf blight was isolated by Yoshii, et al¹⁵⁰ from the soil of the diseased rice paddies and affected leaves of Ajitsuke-mura, Mitsui-gun, Fukuoka Prefecture and named OP₁ (abbreviation for Xanthomonas oryzae phage No. 1) by Wakimoto.^{134,136} Its physical and chemical characteristics were clarified. Then, Kuhara, et al¹⁵⁹ isolated a phage with a host-range completely different from that of OP₁ and the Benikonaya strain, showing an affinity with it at Benikonaya, Okawa City, Fukuoka Prefecture. And Yodo, et al¹⁴¹ clarified the characteristics of the phage in question, and it was named OP_{1h} (Host range mutant of OP₁). According to the investigation conducted by Togami, et al,⁷⁰ there is wide distribution of OP_{1h} and strains showing affinity with it in the coastal area along the Ariake Sea, Kyushu. Furthermore, Wakimoto¹⁴¹ discovered OP_{1t}, a mutant of OP₁.

In contrast to this, the author, using OP_{1h} isolated by Kuhara, et al, and the Benikonaya strain (hereafter referred to as B strain) with an affinity to it, OP₁ isolated by Yoshii and the Shinjo strain (referred to as S strain) with an affinity to it, collected the affected leaves and irrigation water in the area of occurrence of rice bacterial leaf blight in the Hokuriku District. The following experiments were made in order to test the phages contained in them and to find out indirectly the distribution of strains.

1) Test Method

The test method of the sample phages was the plaque formation method, and at the same time, the shapes of the plaques were observed. For collecting irrigation water samples, sterilized test tubes or polyethylene bottles were used as containers. Vinyl envelopes were used for collecting affected leaves. For testing and isolating phages from samples, irrigation water was mixed with bacterial suspension

Table 22
Results of the Testing of Phages in Hokuriku
District by S & B Strains

Sample No.	Place of Collection	Rice Variety	Test Bacteria	
			Shinjo Strain	Benikomaya Strain
Affected Leaves				
1	Ozeki, Niitsu Municipality, Niigata Prefec.	Unknown	+	+
2	Shirane-cho, Nakakamabara-gun, Niigata Prefec.	Echigo early maturing	-	-
3	Nosudo, Shirane-cho, Nakakamabara-gun, Niigata Pref.	(barnyard grass)	-	-
4	Koshiji-cho, Nishima-gun, Niigata Pref.	Sanin #52	+	-
5	Sugawa, Mishima-gun, Niigata Prefec.	Unknown	-	+
6	Sanjo Municipality, Niigata Prefec.	Unknown	-	-
7	Agricultural Experimental Station, Nagaoka, Niigata Prefec.	Echigo early maturing	+	-
8	Higoshi, Nagaoka Municipality, Niigata Prefec.	Unknown	-	+
9	Tokaichi Municipality, Niigata Prefec.	"	+	-
10	Tokaichi Municipality, Niigata Prefec.	"	+	+
11	Hoonji-cho, Kakizaki-cho, Nakakeijo-gun, Niigata Pref.	Fujisaka No. 5	+	-
12	"	Norin No. 16	+	-
13	Osino, Takada Municipality, Niigata Prefec.	Unknown	+	-

14	Agricultural Experimental Station, Takada Municipality, Niigata Pref.	Leersia oryzoides (Linn) Sw.	+	-
15	"	Kinnanpu	+	+
16	Suzawa Aomi-cho, Nishi-keijo-gun, Niigata Pref.	Norin No. 1	-	+
17	Itooukawa Municipality, Niigata Pref.	(Grass)	+	-
18	"	Unknown	-	-
19	Nikkaichi, Akamaru, Fukuoka-cho, Nishi-tonami-gun, Toyama Pref.	Kiyosumi	+	-
20	Oe, Nishi-tonami-gun, Toyama Pref.	Unknown	+	+
21	Tsuchiya, " " " "	Norin No. 29	-	-
22	Kamojima, Tonaka-cho, Nishi-tonami-gun, Toyama Pref.	Kinnanpu	+	-
23	Todashi-cho, Nishi-tonami-gun, Toyama Pref.	Unknown	+	-
24	Hokuden Farm, Kureba-cho, Cuke-gun, Toyama Pref.	Unknown	+	-
25	Okubo, Suberibaya, Yoshino-cho, Suberikawa-gun, Toyama Pref.	Echigo Nebari	+	-
26	Toyama Agricultural Station, Toyama Pref.	Unknown	+	-
27	Ishida, Kurobe Municipality, Toyama Pref.	Shirogane	+	-
28	"	Shinyu	+	-
29	Kitagata, Kawakita-gun, Ishikawa Pref.	Haya Norin	+	-

30	Saita, Norimoto-cho, Kawakita-gun, Ishikawa Pref.	Koshiji early maturing	+	-
31	Ima-cho, Kawakita-gun, Ishikawa Pref.	" "	+	-
32	Nanukaichi-cho, Kaga Municipality, Ishikawa Pref.	Norin No. 32	+	-
33	Ogio-cho, Daisei-ji, Kaga Municipality, Ishikawa Pref.	Koshiji early maturing	+	-
34	Matsutomo-cho, Ishikawa-gun, Ishikawa Pref.	Okuba No. 225	+	+
35	Kushi-cho, Komatsu Municipality, Ishikawa Pref.	Takane Nishiki	+	-
36	" "	Okuba No. 225	-	+
37	Kashimamichi-cho, Hanesase Municipality, Ishikawa Pref.	Haya Norin	+	+
38	" "	Towada	+	-
39	Higashi Babo, Kashima-gun, Ishikawa Pref.	Okuba No. 225	+	-
40	Ozaki, Kashima-gun, Ishikawa Pref.	Koshiji, early maturing	+	-
41	Matsunami-cho, Famasu Municipality, Ishikawa Pref.	Norin No. 6	+	-
42	" "	Takane Nishiki	+	-
43	" "	Honen early maturing	+	-
44	Sosogi, Ichino-cho, Wajima, Ishikawa Pref.	Unknown (late maturing)	-	+
45	" "	Unknown (intermediate maturing)	+	+
46	Ishikawa Agricultural Experimental Station, Ishikawa Pref.	Unknown	+	-
47	Bessho, Sanbo-cho, Sanbo-gun, Fukui Pref.	Senbonasahi	-	-
48	Nishida, Sanbo-cho, Sanbo-gun, Fukui Pref.	Kogane-nami	+	+

49	Kishida, Sanbo-cho, Sanbo-gun, Fukui Pref.	Etsunan No. 22	-	+
50	Pessho, Sanbo-cho, Sanbo-gun, Fukui Pref.	Kinappu	+	+
51	Ozeki, Sakai-mura, Sakai-gun, Fukui Pref.	Koshiji early maturing	+	+
52	Yamamuro, Kanetsu-cho, Sakai-gun, Fukui Pref.	Chusei Shinsenbon	-	+
53	Fusada, Kawanichi-mura, Sakai-gun, Fukui Pref.	Kotobukimochi	+	+
54	Sakimura Nishi, Sakai-gun, Fukui Pref.	Fukuminori	+	+
55	Tsuchishinjo, Sakai-mura Nishi, Sakai-gun, Fukui Pref.	"	-	-
56	Koruoka-cho, Sakagura, Sakai-gun, Fukui Pref.	"	+	+
57	Kamikomori, Kasuga-cho, Sakai-gun, Fukui Pref.	Chusei Shinsenbon	-	+
58	" " " " " " " "	Unknown	+	+
59	Mihana-cho, Fukui Pref.	"	-	+
60	Ariake-cho, Sabae Municipality, Fukui Pref.	Fukuminori	-	+
61	Shinzei-cho, Sabae Municipality, Fukui Pref.	Norin No. 23	+	+
62	Miyazaki, Takabana-cho, Omeshi-gun, Fukui Pref.	Norin No. 30	-	-
63	Komatsu, Iakeo Municipality, Fukui Pref.	Chusei Asahi	+	-
64	Kasuga, Ono Municipality, Fukui Pref.	Sanin No. 17	+	+
65	Ashibane-cho, Ashibane-gun, Fukui Pref.	Kinnanpu	-	+
66	Suenotani, Miyazaki-mura, Nioi-gun, Fukui Pref.	"	-	-

		Sasashigure	
67	Shonai Branch, Yamaga Agricultural Experimental Station, Yamagata Pref.	-	+
<hr/>			
Irrigation Water	1 Ozeki, Niitsu Municipality, Niigata Pref.	+	+
	2 Shirane-cho, Naka-kamabara-gun, Niigata Pref.	+	-
	3 Umenoki, Naka-kamabara-gun, Niigata Pref.	-	+
	4 Niigata Agricultural Experimental Station, Nagaoka Municipality, Niigata Pref.	+	+
	5 Koshiji-cho, Nagaoka Municipality, Niigata Pref.	+	+
	6 Aono Tomoji, Naoetsu Municipality, Niigata Pref.	+	+
	7 Nokuriku Agricultural Experimental Station, Niigata Pref.	+	+
<hr/>			
8	Kurobe Municipality, Toyama Pref.	+	-
9	Tachiyama-cho, Nakaarakawa-gun, Toyama Pref.	+	-
10	Toyama Agricultural Experimental Station, Toyama Pref.	+	-
11	Araminato Municipality, Toyama Pref.	+	-
12	Korimi Municipality, Toyama Pref.	+	+
13	Fukuoka-cho, Nishi-tonami-gun, Toyama Pref.	+	+

14	Tamasu Municipality, Ishikawa Pref.	+	-
15	Higashi-baba, Kashima-gun, Ishikawa Pref.	+	+
16	Morimoto-cho, Kawakita-gun, Ishikawa Pref.	-	-
17	Komeizumi-cho, Kanazawa Municipality, Ishikawa Pref.	-	-
18	Daiseiji, Kaga Municipality, Ishikawa Pref.	+	-
<hr/>			
19	Ozeki, Sakai-gun, Fukui Pref.	+	+
20	" "	+	+
21	Fukui Agricultural Experimental Station, Fukui Pref.	-	+
22	Ono Municipality, Fukui Pref.	+	+
23	Nihama-cho, Sanbo-gun, Fukui Pref.	+	-

in order to form plaques. The affected leaves were placed in wide-mouth jars. After adding a suitable volume of sterilized water, the jars were shaken. Then the liquid of isolated phages was mixed with the bacterial suspension for flat cultivation to form plaques.

2) Test Results and Observations

As shown in Table 22, phages were detected from the affected leaves and irrigation water collected in various places in the Hokuriku District. Some phages showed bacterial reaction to either indicator strains, S strain or B strain, while some showed bacterial reaction to neither, and some showed bacterial reaction to both. It seemed, when there was no bacterial reaction at all, no phages happen to be present in samples. But in the case of the simultaneous bacterial reactions of phages in samples to S and B strains, it was estimated that OP₁ and OP_{1h} were mixed in the samples or new phages were present with different affinity from OP₁ or OP_{1h}.

2. The Relationship Between the Pathogen of Rice Bacterial Leaf Blight in the Hokuriku District and Phages¹⁵⁵

From the tests described in the preceding section, it seemed that there were phages with simultaneous bacterial reactions to S strain and B strain in the Hokuriku District (hereafter these will be referred to as SB phage and I phage as temporary names until the abbreviation of OP₂ is formally decided upon). Therefore, in order to confirm this, affected leaves and irrigation water were collected at places shown in Table 23, and the bacteria and phages were isolated and their affinity was investigated.

1) Test Method.

The method described in 1. was followed.

2) Places of Isolation of Strains.

The places of sample collection of strains for this experiment are shown in Table 23.

3) Places of Collection of Phages

The places of collection of sample phages (irrigation water) for the bacterial reaction test are shown in Table 24.

Table 23

Places of Collection and Varieties of Affected
Leaves for the Isolation of Bacteria

Strain No.	Place of Collection of Affected Leaves	Variety
H5801	Ozoki, Niitsu Municipality, Niigata Pref.	Echiei
H5802	Muramatsu-cho, Naka-kamabara- gun, Niigata Pref.	Unknown
H5803	Sasabori, Itsuizumi Municipality Niigata Pref.	"
H5804	Shinkai-mura, Naka-kamabara-gun, Niigata Pref.	"
H5805	Ryosen-mura, Naka-kamabara-gun, Niigata Pref.	Kinnanpu
H5806	Shirane-cho, Naka-kamabara-gun, Niigata Pref.	Unknown
H5807	Branch Prefectural Agricultural Experimental Station, Naka- kamabara-gun, Niigata Pref.	Koshiji early maturing
H5808	Sanjo Municipality suburb, Niigata Pref.	Unknown
H5809	Prefectural Agricultural Exper- imental Station, Nagaoka, Niigata Pref.	Yachikogane
H5810	Koshiji-cho, Mishima-gun, Nii- gata Pref.	Sanin No. 52
H5811	Hikoshi-cho, Nagaoka Munici- pality, Niigata Pref.	Unknown
H5812	Tokaichi Municipality, Niigata Pref.	"
H5813	" " "	"
H5814	Kakizaki-cho, Naka-keijo-gun, Niigata Pref.	Norin No. 16
H5815	" "	Fujisaka No. 5
H5816	Shitaborinouchi, Takada Munici- pality, Niigata Pref.	Unknown
H5817	Ogino, Takada, Niigata Pref.	Norin No. 29
H5818	Nokuriku Agricultural Exper- imental Station, Takada, Niigata Pref.	Kinnanpu
H5819	" " " "	Echigo Nebari
H5820	" " " "	Ginchu
H5821	Aomi-cho, Nishi-keijo-gun, Niigata Pref.	Norin No. 1
H5852	Ozawa Niitsu Municipality, Niigata Pref.	Unknown

H5853	Nagaoka Municipality, Niigata Pref.	Unknown
H5822	Tsuzawa, Itouokawa Municipality, Niigata Pref.	Weed
H5823	Ishida, Kurobe Municipality, Toyama Pref.	Shinyu
H5824	Okubo, Suberibaya, Suberikawa Municipality, Toyama Pref.	Echigo Nebari
H5825	Fukuoka-cho, Tonami-gun, Toyama Pref.	Kiyosumi
H5826	" " "	Norin No. 29
H5827	Tonaka-cho, Tonami-gun, Toyama Pref.	Kinnanpu
H5828	Machino-cho, Wajima Municipality, Ishikawa Pref.	Unknown (late maturing)
H5829	Higashi-baba, Kashima-gun, Ishikawa Pref.	Ou No. 225
H5830	Ozaki, Kashima-gun, Ishikawa Pref.	Koshiji early maturing
H5831	Kashimamichi-cho, Hanesase Municipality, Ishikawa Pref.	Haya Norin
H5832	" " "	" "
H5833	" " "	" "
H5834	" " "	Towada
H5835	" " "	"
H5836	Ishikawa Prefectural Agricultural Experimental Station	Unknown
H5837	Saita, Morimoto-cho, Kawakita-gun, Ishikawa Pref.	Koshiji early maturing
H5838	Imacho, Morimoto-cho, Kawakita-gun, Ishikawa Pref.	" "
H5839	Matsutomo-cho, Ishikawa-gun, Ishikawa Pref.	" "
H5840	Kushi-cho, Komatsu Municipality, Ishikawa Pref.	Ou No. 225
H5841	" " "	Takane Nishiki
H5842	Hata-cho, Daiseiji, Kaga Municipality, Ishikawa Pref.	Ou No. 225
H5843	Ogio-cho, Daiseiji, Kaga Municipality, Ishikawa Pref.	Unknown
H5844	Yoneizumi-cho, Kanazawa Municipality, Ishikawa Pref.	Koshiji early maturing
H5845	Ozeki, Sakai-mura, Sakai-gun, Fukui Pref.	" "
H5846	Arisada-cho, Sabae Municipality, Fukui Pref.	Fukuminori
H5847	Ashiba-mura, Ashiba-gun, Fukui Pref.	Kinnanpu

H5848	Komatsu, Takeo Municipality, Fukui Pref.	Sosei Asahi
H5849	Bessho, Sanbo-cho, Sanbo-gun, Fukui Pref.	Senbon Asahi
H5850	" " "	Koganenami
H5851	Miyazaki, Takahama-cho, Omeshi-gun, Fukui Pref.	Norin No. 30

Table 24

Places of Isolation of Sample Phages

Place	Strains Used for Phage Isolation			
	Shinjo Strain	Benikonaya Strain	H5801	H5831
Ozeki, Niitsu Municipality Niigata Pref.	+	+	+	-
Shirane-cho, Nakakamabara- gun, Niigata Pref.	+	+	+	-
Umenoki, Nakakamabara-gun, Niigata Pref.	+	+	-	-
Agricultural Experimental Station, Nagaoka Munici- pality, Niigata Pref.	+	+	+	-
Koshiji-cho, Nagaoka Municipality, Niigata Pref.	+	+	+	-
Hokuriku Agricultural Exper- imental Station, Takada Municipality, Niigata Pref.	+	+	+	-
Higashi Baba, Kashima-gun, Ishikawa Pref.	+	+	+	-
Kashimamichi-cho, Hanesase Municipality, Ishikawa Pref.	+	+	+	+
Ozeki, Sakai-mura, Sakai- gun, Fukui Pref.	+	+	+	-
" " "	+	+	+	-

Note: All phages were isolated from irrigation water, and those with asteriks* were selected as samples.

4) Test Results and Observations

The mutual bacterial reactions between the 53 isolates as shown in Table 23, and the 17 isolate phages shown in Table 24 are shown in Table 25.

As shown in Table 25 the 17 sample phages used in this experiment showed various bacterial reactions with the isolate strains from several places in the Hokuriku District. However, from their affinities with several strains, they can be classified, as shown in the upper part of Table 25, into S, SB, B and I (provisional name) with different host ranges. Of these, the S phage group, so tentatively termed by the author, corresponds to OP₁ in terms of its reaction to the S strain. The B phage group is estimated to correspond to OP_{1h} in terms of its reaction to the B strain. And SB and I phage** groups are clearly phages with a different host range. Of these, the I phage group has a somewhat wider host range and its plaque shape is pin-hole type. Therefore, this was regarded as a kind of plaque shape mutant.

** I phages were formally named OP²_m with an abbreviation from the experiments described later. At this point, its tentative name is used.

Table 25

Results of Bacterial Reaction Tests Between
the Isolated Bacteria and the Phages from
Several Places in the Hokuriku District

菌株 No.	S*								SB†				B**				I††	菌 型 判 別
	七 尾 S	坂 井 S	大 岡 S	白 根 S	高 田 S	梅 木 S	七 尾 N	坂 井 N	坂 井 N	坂 井 B	梅 木 B	長 岡 B	七 尾 B	大 岡 B	長 岡 B	白 根 B	七 尾 I	
H5801	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	A
H5802	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B
H5803	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	A
H5804	+	+	+	+	+	+	+	+	+	+	+	△	-	-	-	-	+	A
H5805	+	-	+	+	+	+	+	+	+	△	+	△	-	-	-	-	+	A
H5806	+	+	+	+	+	+	+	+	+	+	+	△	-	-	-	-	+	A
H5807	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	A
H5808	-	-	+	-	-	-	-	-	+	+	+	+	+	+	-	-	+	B?
H5809	+	+	+	+	+	+	+	+	+	△	+	△	-	-	-	-	+	A
H5810	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	C
H5811	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	C
H5812	+	+	+	+	+	+	+	+	+	△	-	-	-	-	-	-	+	A
H5813	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C
H5814	+	+	+	-	+	?	+	+	+	-	+	-	-	-	-	-	+	A
H5815	+	+	+	+	+	+	+	+	+	-	+	△	-	-	-	-	+	A
H5816	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	-	+	AB?
H5817	-	+	+	-	+	-	+	-	+	+	-	-	-	-	-	-	+	A
H5818	+	+	+	+	+	+	+	+	+	△	-	△	-	+	-	-	+	AB?
H5819	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	-	+	AB?
H5820	+	+	+	+	+	+	+	+	△	+	+	-	-	-	-	-	+	A
H5821	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	B?
H5822	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B
H5852	+	+	+	+	+	?	+	+	+	+	+	-	-	-	-	-	+	A
H5853	+	+	+	-	+	-	+	+	+	+	+	-	-	-	-	-	+	A
H5823	+	+	+	+	+	+	+	+	+	△	-	-	-	-	-	-	-	D
H5824	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	C
H5825	-	+	+	-	-	-	+	-	+	-	+	△	-	-	-	-	+	A?
H5826	-	-	+	-	-	-	+	-	+	-	+	-	-	-	-	-	-	D?
H5827	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	D

H5828	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B
H5829	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	A
H5830	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	A
H5831	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	C
H5832	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-	C
H5833	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	A
H5834	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	A
H5835	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	AB
H5836	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	E
H5837	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	A
H5838	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	A
H5839	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C
H5840	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B
H5841	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	A
H5842	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B
H5843	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	E
H5844	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	A
H5845	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	A
H5846	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B
H5847	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B
H5848	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	+	A
H5849	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B
H5850	-	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	+	A
H5851	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B
新庄園	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	A
"	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	A
紅粉園	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B
"	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	B

Note: *OP₁
 **OP_{1h} Seem to have the same characteristics.

+ Phages with bacterial action in S and B strains

++ Phages that form pin-hole plaques

O Plaques are very thin.

• Thin

• Somewhat thin

△ Slight reaction

Legend: 1) Strain No.; 2) Nanao; 3) Sakai;
4) Ozeki; 5) Shirane; 6) Takada; 7) Umenoki;
8) Nagaoka; 9) Lyso-type; 10) Shinjo strain;
11) Denikonaya strain.

3. Isolation of OP₂ (SE and I phages)¹⁵²

In the foregoing 1. and 2. a description of SB and I phages which have different host ranges and plaque shapes from those of OP₁ and OP_{1h} has been given. Since groups of plaques were offered for testing, it is possible to think that OP₁ and OP_{1h} phages were mixed in these phages. For this reason, by repeating single plaque isolation for distinguishing mixed phages, SB and I phages with new host ranges were separated.

1) Experimental Method

With S and B strains as indicators, a certain volume of irrigation water in the vicinity of the Hokuriku Agricultural Experimental Station was collected on 30 June 1959. Plaques were formed by the usual method, and OP₁ and SB phages were separated from the sample by the isolation process shown in Figure 4. OP_{1h} was not included in the irrigation water used for this experiment.

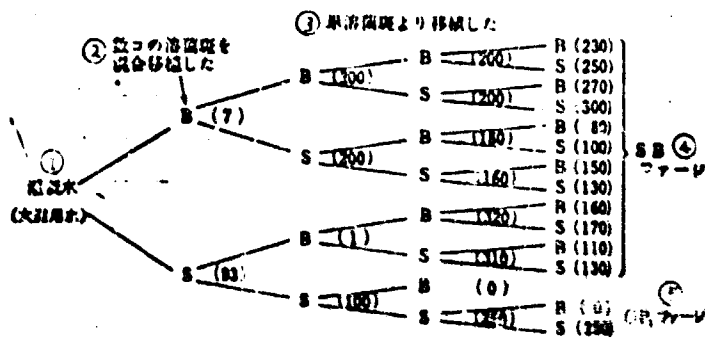


Fig. 4 Isolation Process of OP₂ Phages (SB)

Legend: 1) Irrigation water; 2) Several plaques were mixed and transplanted; 3) Transplanted from single plaques; 4) SB phage; 5) OP₁ phage.

Note: S = Shinjo strain
B = Benikonaya strain
Figures in () are the number of plaques.

Next, I phages were isolated by repeating single plaque isolation using the fact that the plaque shape in bacteria with affinity is the pin-hole type as the guide.

2) Experimental Results

The isolation process of SB phages is as shown in Figure 4.

Furthermore, I phages were isolated from the phages separated from the affected leaves collected at Ishida, Kurobe Municipality, Toyama Prefecture in 1959, with the H5921 strain as the indicator and the plaque shape as the guide, by repeating the process several times.

3) Confirmation of Isolation

The affinities of SB phages obtained with the isolation process shown in Figure 4 and I phages produced by repeated separation of the plaque were tested. Both groups showed simultaneous bacterial reactions to both strains. From this it was confirmed that SB and I phages have different host ranges from those of OP₁ and OP_{1h}.

4. The Host Range of OP₂ (SB and I phages)¹⁶²

1) Experimental Method

Tests were made to determine the host ranges of S, B, H5806, H5823, H5822, H5839, H5831, with different affinity reactions. As seen from the results shown in Table 25, H5921, H5902, H5913 were newly isolated in 1959, and H5925 was donated by the Agricultural Technology Research Center, for a total of 11 strains by the use of OP₁, OP_{1h}, OP_{1h2}^{*}, SB and I phages. Each test strain was slope cultivated in the semi-synthetic culture medium at 28°C for three to four days. Bacterial suspension, produced by adding 2 ml of sterilized water into the test tube and the certain volume of solution of the aforementioned phages, was added to this. Then about 3 ml of melted semi-synthetic agar culture medium (cooled to 55°C) was added to form flat plates. In about 20 hours the presence of plaques and plaque shapes were investigated.

* These phages were isolated by the Division of Bacteriological Research, Department of Pathology, Agricultural Technology Research Center in 1954. They are identical with OP₁ in morphological and serological terms. But because they have different host ranges, they have been named OP_{1h2} host range mutant number 2 of OP₁.

2) Experimental Results and Observations

As shown in Table 26, five kinds of test phages showed different affinity reactions to each strain. Thus the affinity relationship between these phages and strains can be classified into several types of phages with different host ranges. According to the results of this experiment, SB and I phages show the completely same host range, somewhat wider than that of OP₁, OP_{1h}, OP_{1h2}. And the plaques formed by SB phages are somewhat smaller than the plaques of other phages. It was confirmed that I phages in particular are characterized by formation of pin-hole type plaques in the bacterial reactions to certain strains.

Table 26

Host Ranges of Phages of Pathogen of Rice
Bacterial Leaf Blight

Lyso-type	Strain Number	OP ₁	OP _{1h}	OP _{1h2}	SB	I
A	Shinjo strain	+	-	+	+	+
	H5306	+	-	+	+	+
	H5921	+	-	+	+	+
	(A') H5823	+	-	+	+	+
B	Benikonaya strain	-	+	+	+	+
	H5822	-	+	+	+	+
C	H5839	-	-	-	-	-
	H5902	-	-	-	-	-
D	H5925	-	-	+	+	+
E	H5913	-	-	-	+	+
	H5831	-	-	-	+	+

5. Serological Reaction of OP₂ (SB and I phages)¹⁶²

The following experiment was conducted in order to ascertain whether the SB and I phages isolated by the author were serologically identical with the known OP₁, OP_{1h}, and OP_{1h2}.

1) Experimental Method

Using OP₁, OP_{1h} donated by the Kyushu Agricultural Experimental Station, OP_{1h2} contributed by the Mukai Research Section, Department of Pathology of the Agricultural Technology Center, and OP₁, OP_{1h}, SB and I phages isolated by the author, each phage fluid was injected into house rabbits at 3-4 day intervals, between 20 October and 24 November 1959, and thus anti-serum was made. The first four inoculations consisted of 3-6 ml each in the abdominal cavity, and then 1 ml venous injections at first, and then 3 ml six times. By the usual method anti-serum was made. Using the aforementioned seven kinds of anti-serums, each phage was cross-reacted by the neutralization method and their inactivity was divided into the 0 minute (2-3 seconds), five minute, and 10 minute stages. After the elapse of the designated time, sterilized water was added for dilution. Their reactions were suspended and their inactivity was tested by the usual method and by the formation of plaques.

2) Test Results and Observations

The results of the serological reactions by the aforementioned method are shown in Tables 27 and 28. However because the anti-serum of OP_{1h2} was produced by the Department of Medicine, Niigata University, the experiment on it was conducted separately.

As shown in Table 27, SB and I phages have quite different serological reactions in comparison with OP₁ and OP_{1h} which have been tested. That is, the anti-serum of OP₁ and OP_{1h} mutually or alternately inactivate OP₁ and OP_{1h}, as has been shown before; but the anti-serum of OP₁ does not inactivate SB and I phages. Further it was seen that the anti-serum of OP_{1h} considerably inactivates SB and I phages. However, in contrast to this, while the anti-serums of SB and I phages mutually or alternately inactivate SB and I phages, but they do not inactivate OP₁ and OP_{1h}. This proves, in serological terms, that SB and I phages are identical, and are quite different from OP₁ and OP_{1h}.

Table 27
Serological Reaction of Each Phage Group
(number of plaques)

Anti-serum	Reaction Time (minute)	Hokuriku District OP ₁	Kyushu District OP ₁	Hokuriku District OP _{1h}	Kyushu District OP _{1h}	SB	I
OP ₁ Hokuriku District	0 5 10	0 0 0	0 0 0	0 0 0	1 0 0	305 263 270	177 160 164
OP ₁ Kyushu District	0 5 10	1 0 0	4 0 0	42 4 0	11 0 0	224 248 248	170 172 165
OP _{1h} Hokuriku District	0 5 10	20 0 0	35 0 0	10 0 0	5 0 0	113 79 24	69 10 5
OP _{1h} Kyushu District	0 5 10	1 0 0	13 0 0	0 0 0	0 0 0	112 2 0	53 1 0
SB	0 5 10	161 175 220	264 221 234	558 323 352	230 248 184	0 0 0	3 0 0
I	0 5 10	204 260 172	274 288 237	- 264 257	408 237 145	0 0 0	0 0 0
Standard District (Sterilized water)		316	153	344	204	242	168

Table 28

Relationship Between OP_{1h2} Phages and the
Anti-serum of Each Phage (1960)

① 血清	② OP ₁ (北 陸)			③ OP ₁ (九 州)			OP _{1h} (北 陸)			OP _{1h} (九 州)			④ OP _{1h2} (農技研)			SB (北 陸)			I (北 陸)			⑤ 標準
⑥ 反応時間(分)	0	5	10	0	5	10	0	5	10	0	5	10	0	5	10	0	5	10	0	5	10	殺菌水
⑦ フォージ OP _{1h2}	43	0	0	46	0	0	11	16	0	12	10	0	12	0	0	168	148	110	161	154	102	161

[Legend]: 1) Anti-serum; 2) Hokuriku District; 3) Kyushu District; 4) Agricultural Technology Research Center; 5) Standard sterilized water; 6) Reaction time (minute); 7) Phage.

6. Inactivation Temperature of OP₂ (SB and I phages)¹⁶²

1) Experimental Method

SB and I phages were suspended in the semi-synthetic culture medium (pH 6.5) and sterilized water (pH 6.4), and were then poured into test tubes, 1 ml each, and the test tubes were dipped for 10 minutes in the thermostat tank adjusted to temperatures from 52°C to 72°C. After this treatment, they were cooled by cold water (about 10°C), and flat cultivated by mixing with the bacteria suspension of H5813 strain (lyso-type E), and H5921 strain (those are types that form pin-hole type plaques in their reaction to I phages) obtained by the usual method. They were preserved in a thermostat at 28°C, and the number of plaques was counted in 16 hours. The standard temperature of this experiment was set lower than room temperature (23°C).

2) Experimental Results and Observations

The results of the experiment conducted by the aforementioned are shown in Table 29. This can be further demonstrated in Figure 5.

Table 29

Inactivation Temperatures of SB and I Phages
(The mean values of three repetitions on
27 January 1960)

① 処理温度 (°C)	② SB フ ァ ー ジ				③ I フ ァ ー ジ			
	④ 殺 菌 水		⑤ 馬 鈴 薯 半 合 成		殺 菌 水		馬 鈴 薯 半 合 成	
	⑦ 溶菌斑数	⑧ (%) 標準対比	溶菌斑数	標準対比 (%)	溶菌斑数	標準対比 (%)	溶菌斑数	標準対比 (%)
⑥ 標準区 (室温)	206	100	205	100	149	100	146	100
52	200	97.1	210	102.4	127	85.2	124	84.9
54	202	98.1	198	96.6	135	90.6	106	72.6
56	198	96.1	187	91.2	129	86.6	105	71.9
58	186	92.1	191	93.2	113	75.8	61	41.8
60	164	79.6	163	79.5	103	69.1	13	8.9
62	150	72.8	145	70.7	91	61.1	3	2.1
64	113	55.7	64	31.7	63	42.3	0.3	0.2
66	80	38.8	6	2.9	29	19.5	0.3	0.2
68	27	7.8	0.3	0.1	0.7	0.5	0.7	0.5
70	0.3	0.1	0.7	0.3	0	0	0	0

[Legend]: 1) Treatment temperature; 2) SB phage; 3) I phage; 4) Sterilized water; 5) Semi-synthetic potato; 6) Standard area (room temperature); 7) Number of plaques; 8) Ratio over the standard (%).

As shown in Table 29 and Figure 5, SB phages could be completely inactivated even by a 10 minute treatment at 70°C, and I phages were viable up to 68°C. The temperature ranges for inactivating 50% were 64-66°C for SB phages in sterilized water, and 62-64°C in the semi-synthetic culture medium, for I phages, 62-64°C in sterilized water, and 56-68°C in the semi-synthetic culture medium.

The foregoing results, when compared with the report made by Wakimoto¹³⁴ that the complete inactivation of OP₁ and OP_{1h} takes place in the 58°C or 60°C range, reveal that SB and I phages have high heat-resistance.

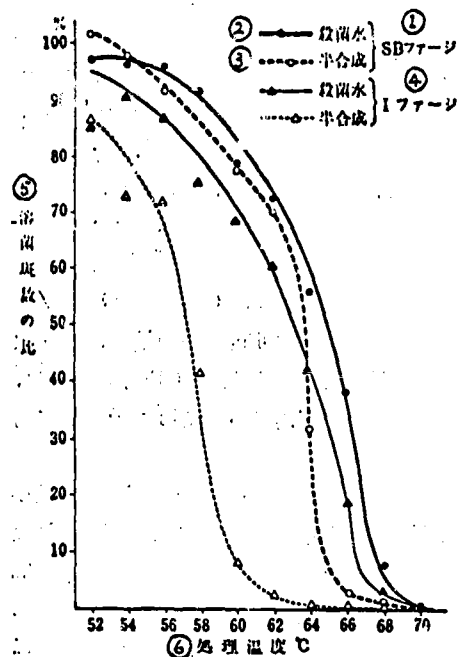


Fig. 5 Test Results on the Inactivation Temperatures of SB and I Phages

[Legend]: 1) SB phages; 2) Sterilized water; 3) Semi-synthetic; 4) I phages; 5) Percentage of the number of plaques; 6) Treatment temperature.

7. One-step Growth Experiments of OP₂ (SB and I phages)¹⁶²

One strain each, as will be described later, was selected from lyso-type A, B, D, and E strains with affinities to SB and I phages, in order to conduct an experiment on the process of the one-step growth formed between SB and I phages, and each strain.

1) Experimental Method¹³⁶

Test sample phages are SB and I phages, and the test sample strains are as follows:

Lyso-type A strain	H5801, H5921 strains
Lyso-type B strain	Benikonaya strain
Lyso-type D strain	H5925 strain
Lyso-type E strain	H5913 strain

Bacteria obtained by the single colony isolation of the test sample strains repeated twice were used as adsorbed bacteria and the plaque indicator. For the experiment, a 9 ml bacteria suspension in which the bacteria slope cultivated on the semi-synthetic agar culture medium for 24 hours would be about 10^8 /ml on the Cavfch culture medium (will be described later), and 1 ml of SB phage liquid with the density adjusted at about 10^8 /ml beforehand, was prepared. This suspension and phage liquid were simultaneously poured into small flasks for adsorption and allowed to set for five minutes. Next, the anti-serums of SB and I phages (those with the anti-bodies of the cultivated bacteria completely eliminated) were added in order to inactivate free phages. In five minutes, they were given a 10^{-1} dilution in three stages on the Cavfch culture medium. From the last growth tube, a certain volume (0.1 ml and 0.01 ml each) was transferred to the suspension of the indicator strain and then poured into and preserved in the thermostat at 27-28°C, for about 20 hours. The numbers of plaques formed were counted, and the process of their one-step growth was investigated. The growth tube was used for the experiment by placing it in the thermostat water tank at 29°C.

2) Experimental Results

a) One-step growth experiments of SB phages.

The one-step growth process of SB phages against A, B, D, and E strains are shown in Tables 30-35.

b) One-step growth experiments of I phages.

The one-step growth process of I phages against A, B, and E strains are shown in Tables 36-40.

3) Summary:

The one-step growth experiments of SB and I phages are summarized in Table 41.

Table 30

One-step Growth Experiment of SB Phages
Against Lyso-type A Strains
(H5801 strain)

③ 稀釈	①時間 ②反復	④ 経 過 時 間 (分)													⑤ 吸着20分 後のfree ファージ
		40	50	60	70	80	90	100	110	120	130	140	150	160	
0.1ml	I	7	7	7	11	44	64	77	124	176	182	184	128	174	9
	II	12	16	15	12	51	79	22	98	132	156	183	330	120	13
	⑥ III	8	7	7	8	19	24	82	106	166	174	185	127	182	6
	平均	9	10	9.7	10	38	56	60	109	158	171	184	195	159	9
0.01ml	I		2	3	2	3	12	27	20	31	46	37	35	52	
	II		0	1	1	3	3	13	24	23	28	24	26	37	
	III		2	0	5	9	8	14	28	28	37	44	41	24	
	平均		1.3	1.6	2.6	5	8	18	24	27	37	35	34	38	

[Legend]: 1) Time; 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free phage after 20 minutes of adsorption; 6) Mean.

Table 31

One-step Growth Experiment of SB Phages
Against Lyso-type A' Strains
(H5921 strain)

③ 稀釈	①時間 ②反復	④ 経 過 時 間 (分)													⑤ 吸着20分 後のfree ファージ
		40	50	60	70	80	90	100	110	120	130	140	150	160	
0.01ml	I	95	73	98	91	68	81	246	338	780	1,096	1,487	1,890	1,770	94
	II	76	73	55	38	78	86	218	286	765	1,200	1,453	1,740	1,890	89
	⑥ III	68	77	85	72	93	167	202	417	901	1,248	1,368	1,770	1,800	33
	平均	80	75	79	67	79	111	222	347	815	1,181	1,438	1,800	1,820	72

[Legend]: 1) Time; 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free phage after 20 minutes of adsorption; 6) Mean.

Table 32

One-step Growth Experiment of SB Phage
Against Lyso-type B Strain
(Benikonaya strain)

③ 稀釈	② 反復	④ 経 過 時 間 (分)													20分後の freeファ- ージ⑤
		40	50	60	70	75	80	85	90	100	110	120	130	150	
0.1ml	I	13	17	14	19	50	125	135	203	266	580	529	584	579	26
	II	19	23	19	22	55	127	173	226	302	442	551	566	502	14
	III	23	19	28	18	25	130	207	138	428	393	448	433	494	13
	⑥ 平均	18	20	20	20	43	127	172	189	332	472	509	528	525	18
0.01ml	I		2	3	2	3	16	18	24	20	50	46	44	46	
	II		3	0	2	4	12	10	29	39	58	58	58	44	
	III		2	2	3	4	13	17	38	42	19	52	56	49	
	平均		2	3	2	4	14	15	30	34	42	52	53	46	

[Legend]: 1) Time; 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free phage after 20 minutes of adsorption; 6) Mean.

Table 33

One-step Growth Experiment of SB Phage
Against Lyso-type D Strain
(H5925 strain)

③ 稀 釈	② 反復	④ 経 過 ⑤ 時 間 ⑥ (分)											20分後のfree ファ-ージ⑦
		45	65	75	85	95	100	110	120	130	140	150	
0.01ml	I	5	6	10	23	36	55	76	167	156	161		5
	II	6	3	11	18	47	43	88	142	173	170		8
	⑥ 平均	5.5	4.5	10.5	20.5	41.5	49.0	82.0	154.5	164.5	165.5		7

[Legend]: 1) Time; 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free phage after 20 minutes of adsorption; 6) Mean.

Table 34

One-step Growth Experiment of SB Phage
Against Lyso-type E Strain
(H5913 Strain)

③ 稀釈	② 反復	④ 経過時間 (分)													⑤ 20分後の free phage アージュ
		40	50	60	65	70	75	80	90	95	100	110	120	150	
0.1ml	I	25	34	40	41	108	135	261	312	656	1,004				23
	II	24	29	28	23	32	106	260	300	656	865				27
	平均	24.5	31.5	34.0	32.0	70.0	120.5	260.5	306.0	656.0	934.5				
0.01ml	I			5	6	4	10	12	28	67	118	82	118	104	
	II			4	2	3	5	8	22	49	106	69	81	—	
	平均			4.5	4.0	3.5	7.5	10.0	25.0	58.0	112.0	75.5	99.5	104.0	

[Legend]: 1) Time; 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free phage after 20 minutes of adsorption; 6) Mean.

Table 35

One-step Growth Experiment of SB Phage
Against Lyso-type E Strain
(H5913 Strain) Part 2

①時間		④経過時間 (分)																		⑤吸着20分後のfreeファージ
③稀釈	②反復	40	50	60	70	80	90	100	110	120	130	135	140	145	150	160	165	170	180	
0.1 ml	I	169	182	195	189	352	720	1,016	1,336	1,320	1,480	1,680	2,240	2,264	2,500					161
	II	125	180	194	188	284	660	656	1,060	1,200	1,560	1,520	—	—	—					128
	III	125	176	179	156	203	690	—	—	—	—	—	—	—	—					—
	②平均	140	179	189	178	280	690	836	1,178	1,260	1,520	1,500	2,240	2,264	2,500					144
0.01 ml	I					25	100	137	153	604	588	654	356	440	508	458	422	328	602	
	II					22	83	107	136	406	432	502	344	420	428	328	403	319	484	
	III					19	69	76	38	312	370	484	322	380	416	316	379	315	480	
	平均					22	84	113	126	441	463	547	341	413	451	367	401	321	522	

[Legend]: 1) Time; 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free phage after 20 minutes of adsorption; 6) Mean.

Table 36

One-step Growth Experiment of I Phage
Against Lyso-type A Strain
(H5801 strain)

③ 稀釈	② 反復	④ 経 過 時 間 (分)													⑤ 吸着20分 後の free ファージ
		30	40	50	60	70	80	90	100	120	130	140	150	170	
0.1ml	I	46	20	28	24	16	34	90	161	205	386	483	509	544	19
	II	22	19	20	22	22	59	95	199	213	599	537	664	519	22
	III	21	23	26	19	20	45	109	199	229	571	495	622	579	20
	⑥ 平均	30	21	25	22	19	46	98	186	216	519	505	598	547	20
0.01ml	I			3	2	0	6	16	16	11	59	44	43	60	
	II			0	1	2	7	23	25	18	43	50	51	77	
	III			2	3	8	12	15	21	24	52	59	60	46	
	⑥ 平均			2	2	3	9	18	21	18	51	51	51	58	

[Legend]: 1) Time; 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free phage after 20 minutes of adsorption; 6) Mean.

Table 37

One-step Growth Experiment of I Phage
Against Lyso-type A' Strain
(H5921 strain)

①時間 ②反復 ③稀釈		④経 過 時 間 (分)																		⑤ 吸着20分 後の free ファージ
		30	40	50	60	70	80	90	100	110	120	130	145	160	170	180	190	200	220	
0.01ml	I	24	32	35	28	39	30	20	43	87	126	319	438	743	941	924	902	n	n	27
	II	16	21	44	31	21	25	28	53	102	203	450	538	809	853	747	1,060	874	n	22
	III	31	23	14	24	27	24	33	69	174	232	435	696	544	864	1,035	910	n	n	26
	⑥ 平均	24	25	31	28	29	26	27	55	121	187	401	557	699	886	902	957	n	n	25

[Legend]: 1) Time; 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free phage after 20 minutes of adsorption; 6) Mean.

Table 38

One-step Growth Experiment of I Phage
Against Lyso-type A' Strain
(H5921 Strain) Part 2

③ 稀釈	② 反復	④ 経 過 時 間 (分)														⑤ 吸着20分 後のfree ファージ
		40	50	60	70	80	90	100	110	120	140	150	160	170	180	
0.1ml	I	134	102	105	109	95	135	101	423	768	1,922	3,103	890	3,800	4,040	90
	II	93	139	121	166	123	113	138	533	883	2,162	2,961	3,740	4,010	n	92
	III	113	114	136	81	118	107	307	296	997	2,198	2,694	3,960	4,160	3,330	157
	⑥ 平均	113	118	121	119	112	118	182	417	883	2,094	2,921	3,863	3,990	3,935	113
0.01ml	I		14	10	14	8	10	15	73	80	99	188	392	437	462	
	II		12	10	9	15	11	14	59	77	94	117	427	474	459	
	III		15	14	15	15	12	44	55	69	59	102	438	442	421	
	⑥ 平均		14	11	13	13	11	24	62	75	84	136	419	451	447	

[Legend]: 1) Time, 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free phage after 20 minutes of adsorption; 6) Mean.

Table 39

One-step Growth Experiment of I Phage
Against Lyso-type B Strain
(Benikonaya Strain)

③ 稀釈	② 反復	④ 経 過 時 間 (分)														⑤ 吸着20分 後のfree ファージ
		40	50	60	70	80	90	100	115	125	135	145	155	165	185	
0.1ml	I	131	103	120	112	174	516	782	1,052	1,652	1,488	3,300	n	n	n	107
	II	140	158	174	166	228	570	880	1,310	1,671	2,365	n	2,730	3,600	n	125
	III	95	112	128	120	235	123	846	1,215	2,084	n	n	2,620	3,100	n	143
	⑥ 平均	122	124	141	133	212	403	836	1,192	1,869	1,897	3,300	2,675	3,350	n	125
0.01ml	I		10	8	12	10	43	51	63	128	149	229	292	376	290	
	II		14	14	13	20	21	68	93	124	166	343	302	362	358	
	III		14	13	15	20	39	59	78	119	181	457	311	320	438	
	⑥ 平均		13	12	13	17	34	59	78	124	165	343	302	352	362	

[Legend]: 1) Time; 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free phage after 20 minutes of adsorption; 6) Mean.

Table 40

One-step Growth Experiment of I Phage
Against Lyso-type E Strain
(H5913 Strain)

①時間 ②反復 ③稀釈		④経過時間 (分)												⑤ 吸着20分 後のfree ファージ	
		50	60	70	80	90	95	100	110	120	130	140	150		170
0.1ml	I	29	23	25	29	23	131	213	355	394	534	776	764	77	22
	II	20	21	17	16	25	118	274	350	443	636	741	712	60	27
	⑥III	27	18	23	18	21	107	259	322	405	521	623	803	797	22
	平均	28	21	22	21	23	119	249	342	414	564	713	759	786	24
0.01ml	I			5	3	4	9	16	46	40	65	67	90	81	
	II			0	1	0	7	19	39	38	60	58	76	77	
	III			2	3	3	7	17	32	37	54	87	62	74	
	平均			2	2	2	8	17	39	38	59	71	76	77	

[Legend]: 1) Time; 2) Repetition; 3) Dilution; 4) Time (minute); 5) Free Phage after 20 minutes of adsorption; 6) Mean.

Table 41

One-step Growth Experiment of SB and I Phages
(in Cavfch Culture Medium)

Phage	Adsorped Strain		Latent Time (minute)	Rise Time (minute)	Average Volume of	
	Strain No.	Lyso-type			First of New Phages	
SB	H5801	A	75	45	18.2-19.6	
	H5921	A'	80	70	24.2	
	Benikonaya	B	70-75	40	25.0-27.0	
	H5925	D	70	45	25.0	
	H5913	E	70-80	40	25.4	
I	H5801	A	70	55	26.3-29.1	
	H5921	A'	90	80	33.8	
	Benikonaya	B	90	70	33.6-35.2	
	H5913	E	75	65	23.5-27.2	
			90	50	32.6-33.0	

8. Morphological Observation of OP₂ (SB and I phages) with an Electron Microscope¹⁶²

The morphological observation of SB and I phages were made using the JEM 5G type electron microscope of the Electron Microscope Division, Department of Medicine, Niigata University, at 80 KV.

The test samples were SB and I phages multiplied by the use of H5921 (lyso-type A strain) and H5913 (lyso-type E strain), OP₁ multiplied by the use of Shinjo strain (lyso-type A strain), OP_{1h} multiplied by the use of Benikonaya strain (lyso-type B strain), and OP_{1h2} multiplied by the use of H5925 strain (lyso-type D strain).

1) Experimental Method

Plaques on the semi-synthetic agar plate were adjusted in such a way that about 200 plaques appeared and by using each phage, transparent bacteria were made by drops of sterilized water. Then after the bacteria grown on the plate were completely bacteriolized, the water on the plate containing thick phage was absorbed by the glass capillary. Then this was diluted 10-100 times and mounted on corrodium mesh for the electron microscope. After it was frozen and dried in a vacuum, it was chrome shadow cast, fluoroscoped, and photographed.

Table 42

Electron Micrograph of SB and I Phages

Phage	Head	Tail	
		Length	Thickness
OP ₁	70 x 70 m μ	150 m μ	15 m μ
OP _{1h}	70 x 70 "	150 "	15 "
OP _{1h2}	70 x 70 "	150 "	15 "
SB	70 x 70 m μ	85 m μ	25 m μ
I	70 x 70 "	85 "	25 "

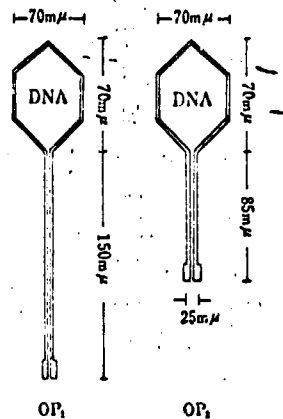


Fig. 6 Morphological Difference Between OP₁ Group Phages and OP₂ Group Phages

2) Observation Results

The morphology of each phage was observed and measured by photographing it with the electron microscope at 10,000 magnifications and then enlarged on printing paper. The results are shown in Table 42 and Figure 6 shows a diagram of the morphological difference between OP₁ group phages and SB and I phages.

In 5, the serological reaction test, the results showed that SB and I phages had markedly different antigen structures and characteristics in the cross-reactions of each antigen phage and each anti-serum. Consequently, it was estimated that they would have different morphologies. In this experiment, SB and I phages showed a different morphology in the tails of phages from those of OP₁, or OP_{1h}, and OP_{1h2}. As shown in Table 42, SB and I phages have shorter tails by 65 m and are thicker by 15 m as compared with those of OP₁, OP_{1h}, or OP_{1h2}. (See photographs 19-23)

9. Affinities of OP₂ (SB and I phages) with Xanthomonas bacteria¹⁷⁰

By using Xanthomonas oryzae and other Xanthomonas bacteria and several other bacteria as test samples, the presence of affinities of OP₂ was examined by the plaque formation method.

1) Test Method

Test Bacteria: two strains each of Xanthomonas compestris, X. citri, X. pruni, X. cucurbitae, X. phaseoli (these were donated by the Department of Pathology, Agricultural Technology Research Center), three strains of X. phaseoli var. sojense (isolated from the soybeans in the field of Niitsu Municipality, Niigata Prefecture, Korimaki-cho, Naka-kamabara-gun, and Takada Municipality), and two strains of two species of Xanthomonas (Yellow bacteria, name unknown, isolated from Setaria viridis Beauv and rice plant in Takada Municipality, Niigata Prefecture)

X. oryzae H5913 (lyso-type E) was used as a check, and the presence of bacterial reaction was examined.

2) Test Results

OP₂ (both SB and I phages) did not show parasiticity to any of the aforementioned bacteria.

10. Summary

The author in 1958, isolated SB and I phages (provisional names) with different host ranges from OP₁ and OP_{1h}, the known pathogenic phages of rice bacterial leaf blight. As a result of this, it was observed that SB phage showed marked differences in the host range, inactivation temperature, serological reaction, and the shape of its phage particles, as compared with those of OP₁ and OP_{1h} phages. It was further demonstrated that SB phage had no parasiticity toward Xanthomonas bacteria, except the pathogen of rice bacterial leaf blight. Consequently, this phage should not be regarded as a host range mutant of OP₁; instead it seems to be proper to call this OP₂, a new phage in the Xanthomonas oryzae phages.

Also I phage has the same or similar characteristics in its serological reaction, inactivation temperature, and SB phage shape. But as described in 4. and as described in the one-step growth experiments in 7., some of the bacteria with affinities show pin-hole plaques in the bacterial reactions against certain strains (See photograph 24), and show marked differences from SB phages in the process of one-step growth. Such a phenomenon indicates the characteristics of a plaque shape mutant. Consequently the author regards it proper to term this phage OP_{2m}, the plaque shape mutant of OP₂.

Section 2. The Lyso-types and Pathogenicity
of the Pathogens of Rice Bacterial Leaf
Blight as Classified by the Host
Range of Phages¹⁶³

Because it was ascertained from the test results as shown in Table 26, that the pathogen of rice bacterial leaf blight can be classified by the host range of each phage into five groups, the author classified them by lyso-types. Then, the author made tests on the relationship between the strains belonging to each lyso-type and the degree of their pathogenicity, and the serological differences of each lyso-type.

1. Classification of Lyso-types¹⁶³

From results shown in Table 26, the pathogens of rice bacterial leaf blight can be classified into five lyso-types according to the host range (affinity types) of the phages. The standards for this classification were further tested by Wakimoto.¹⁴⁵

Table 43

Standards of Lyso-types Classified
by the Host Range of Phages

Lyso-type	Reaction Toward Each Phage
Type A	Strains bacteriolized by OP ₁ , OP _{1h2} , OP ₂ (SB, I)
(Type A')	Shows the same affinity reactions as type A strains; I phage forms pin-hole type plaques and require time for plaque formation (20 hours)
Type B	Strains bacteriolized by OP _{1h} , OP _{1h2} , OP ₂ (SB, I)
Type C	Strains bacteriolized by any of OP ₁ , OP _{1h} , OP _{1h2} , OP ₂ (SB) (Their reaction to I phage is under examination)
Type D	Strains bacteriolized by OP _{1h2} , OP ₂ (SB, I)
Type E	Strains bacteriolized by OP ₂ (SB, I)

2. Lyso-type Classifications and Distribution of the Pathogens of Rice Bacterial Leaf Blight in the Hokuriku District 157, 158, 163

1) Test Method

Lyso-types of 52 isolates of 1958 previously used as test samples (except H5813 strain) and 37 new isolates of 1959-1960, 89 isolates in total, were determined in accordance with the classification standards as described in 1. by using OP₁, OP_{1h}, OP₂, and OP_{2m} produced by single plaque isolation in 1959, and OP_{1h2} donated by the Agricultural Technology Research Center.

Each strain was slope cultivated on the semi-synthetic slope culture medium at 28°C for four days. The phage liquid added to the bacterial suspension of each strain was adjusted so that $nx10^2$ plaques would appear for certain. The strains isolated in 1959-1960 and the places of collection of affected leaves are shown in Table 44.

2) The Results of Determination of Lyso-types of Isolated Strains

The results of the determination of lyso-types of 89 test strains in accordance with the standards described in Table 43 are shown in Table 45.

Table 44

Places of Collection of Affected Leaves from Which Strains were Isolated and Rice Varieties (1959-1960)

Strain No.	Place of Collection of Affected Leaves	Rice Variety
H5901	Tomoji, Aono, Naoetsu Municipality Niigata Pref.	Unknown
H5902	Miwamura Nishiki, Naka-keijo-gun, Niigata Pref.	"
H5903	Shimonoda, Takada Municipality, Niigata Pref.	Kinnanpu
H5904	Tomikawa, Takada Municipality, Niigata Pref.	Sanin No.52
H5905	Matsushiro-cho, Higashi-keijo-gun, Niigata Pref.	Norin No. 29
H5906	" "	Unknown

H5907	Ozeki, Niitsu Municipality, Niigata Pref.	Unknown
H5908	Shimonoda, Takada Municipality Niigata Pref.	Sanin No. 52
H5909	Koshiji-cho, Mishima-gun, Niigata Pref.	Ginmasari
H5910	" " "	Sanin No. 52
H5911	" " "	Hon No. 16
H5912	" " "	Koganemochi
<hr/>		
H5913	Prefectural Agricultural Experimental Station, Toyama Municipality, Toyama Pref.	Shinyu
H5914	Hamagurosaki, Toyama Municipality Toyama Pref.	Unknown
H5915	Mizubashi-cho, Naka-arakawa-gun, Toyama Pref.	"
H5916	" " "	"
H5917	Tachiyama-cho, Naka-arakawa-gun, Toyama Pref.	Kinnanpu
H5918	Kakizawa, Kamiichi-cho, Naka-arakawa-gun, Toyama Pref.	Shirogane
H5919	Maezawa, Kurobe Municipality, Toyama Pref.	"
H5920	Inuyama, Ishida, Kurobe Municipality, Toyama Pref.	Unknown (intermediate maturing)
H5921	Tachino, Ishida, Kurobe Municipality, Toyama Pref.	Koganemochi
H5922	" " "	Sayanukagusa
<hr/>		
H5923	Akumi-gun, Yamagata Pref.	Unknown
H5924	" " "	"
<hr/>		
H6001	Shimotomikawa, Takada Municipality, Niigata Pref.	Sanin No. 52
H6002	Koyasu, Takada Municipality, Niigata Pref.	Unknown
H6003	Urakawa Hara-cho, Higashi-keijo-gun, Niigata Pref.	"
H6004	Kakizaki-cho, Naka-keijo-gun, Niigata Pref.	"
H6005	Kawara-cho, Sado-gun, Niigata Pref.	Yoneyama
H6006	Motoyashiki, Takada Municipality Niigata Pref.	Unknown

H6007	Ozeki, Niitsu Municipality, Niigata Pref.	Unknown
H6008	Maki-cho, Nishi-kamabara-gun, Niigata Pref.	"
H6009	" "	"
H6010	Koyasu, Takada Municipality, Niigata Pref.	Kinnanpu
H6011	Kureha-cho, Toyama Pref.	Unknown
H6012	Shoin, Tamasu Municipality, Ishikawa Pref.	Kagaminori
H6013	Mukogasa, Sanbo-gun, Fukui Pref.	Sayanukagusa

3) Results

As shown in Table 45, the pathogens of rice bacterial leaf blight in the Hokuriku District were classified into 61 lyso-type A, 14 lyso-type B, 7 lyso-type C, 7 lyso-type E. In other words, lyso-type A has the widest distribution. And lyso-type D was not found among the test strains. Although the relationship between each lyso-type and its geographical distribution in the Hokuriku District were not clarified, comparatively many strains among lyso-type A which form pin-hole plaques in bacterial reactions to OP_{2m} were isolated in Toyama Prefecture.

3. The Pathogenicity of the Strains Classified into Each Lyso-type¹⁶³

As to the pathogenicity of rice bacterial leaf blight, Kuhara, et al¹⁵⁹ observed that the isolate from Asakaze, wet rice variety in the Benikonaya area, Okawa Municipality, Fukuoka Prefecture, strongly invaded Ogyoku, a resistant variety. And Kusaba, et al¹⁶² reported that many isolates collected throughout the nation could be classified into two groups: one that strongly invaded both Aichiasahi, a susceptible variety, and Ogyoku, a resistant variety; and another that strongly invaded only Aichiasahi. Seki¹⁰⁹ made similar observations.

Thus it is suggested that there are several groups of pathogens of rice bacterial leaf blight. If the results of determination of lyso-types of strains by the host range (affinity relationship) of phages, as in this experiment should have any relationship with the degree of pathogenicity, the classification and determination of pathogenicity, as well as the ease of the operation for determining lyso-types, will be simplified.

Table 45

Results of Determination of Lyso-types
of Isolated Strains

① 菌株No.	OP ₁	OP _{1b}	OP _{1b2}	SB	I	② 菌型	菌株No.	OP ₁	OP _{1b}	OP _{1b2}	SB	I	② 菌型
H5801	+	-	+	+	+	A	H5847	-	+	+	+	+	B
H5802	-	+	+	+	+	B	H5848	+	-	+	+	+	A
H5803	+	-	+	+	+	A	H5849	-	+	+	+	+	B
H5804	+	-	+	+	+	A	H5850	+	-	+	+	+	A
H5805	+	-	+	+	+	A	H5851	-	-	-	+	+	E
H5806	+	-	+	+	+	A	H5852	+	-	+	+	+	A
H5807	+	-	+	+	+	A	H5853	+	-	+	+	+	A
H5808	-	+	+	+	+	B	H5901	+	-	+	+	+	A
H5809	+	-	+	+	+	A	H5902	-	-	-	-	-	C
H5810	-	-	-	-	-	C	H5903	+	-	+	+	+	A
H5811	-	-	-	+	+	E	H5904	+	-	+	+	+	A
H5812	+	-	+	+	+	A	H5905	+	-	+	+	+	A
H5814	+	-	+	+	+	A	H5906	+	-	+	+	+	A
H5815	+	-	+	+	+	A	H5907	-	+	+	+	+	A
H5816	+	-	+	+	+	A	H5908	+	-	+	+	+	A
H5817	+	-	+	+	+	A	H5909	+	-	+	+	+	A
H5818	+	-	+	+	+	A	H5910	+	-	+	+	+	A
H5819	+	-	+	+	+	A	H5911	+	-	+	+	+	A
H5820	+	-	+	+	+	A	H5912	+	-	+	+	+	A
H5821	-	+	+	+	+	B	H5913	-	-	-	+	+	E
H5822	-	+	+	+	+	B	H5914	+	-	+	+	+	A'
H5823	+	-	+	+	+	A'	H5915	-	-	-	+	+	C
H5824	-	-	-	+	+	E	H5916	+	-	+	+	+	A
H5825	+	-	+	+	+	A	H5917	+	-	+	+	+	A
H5826	-	-	-	-	-	C	H5918	+	-	+	+	+	A'
H5827	+	-	+	±	+	A'	H5919	+	-	+	+	+	A'
H5828	-	+	+	+	+	B	H5920	+	-	+	+	+	A'
H5829	+	-	+	+	+	A	H5921	+	-	+	+	+	A'
H5830	+	-	+	+	+	A	H5922	-	+	+	+	+	B
H5831	-	-	-	+	+	E	H5923	+	-	+	+	+	A
H5832	-	-	-	-	-	C	H5924	-	-	-	+	+	E
H5833	+	-	+	+	+	A	H6001	+	-	+	+	+	A
H5834	+	-	+	+	+	A	H6002	+	-	+	+	+	A
H5835	-	+	+	+	+	B	H6003	+	-	+	+	+	A
H5836	-	-	-	+	+	E	H6004	+	-	+	+	+	A
H5837	+	-	+	+	+	A	H6005	+	-	+	+	+	A
H5838	+	-	+	+	+	A	H6006	+	-	+	+	+	A
H5839	-	-	-	-	-	C	H6007	-	+	+	+	+	B
H5840	-	+	+	+	+	B	H6008	-	+	+	+	+	B
H5841	+	-	+	+	+	A	H6009	+	-	+	+	+	A
H5842	-	+	+	+	+	B	H6010	+	-	+	+	+	A
H5843	-	-	-	-	-	C	H6011	+	-	+	+	+	A
H5844	+	-	+	+	+	A	H6012	+	-	+	+	+	A
H5845	+	-	+	+	+	A	H6013	+	-	+	+	+	A
H5846	-	+	+	+	+	B							

[Legend]: 1) Strain Number; 2) Lyso-type.

1) Test Method

a) Rice Plant Varieties for Determining Pathogenicity: Ogyoku, Akajinriki, Zensho No. 26, Norin No. 27, Asakaze, Kamizeki No. 1, Yachikogane, Norin No. 18, Toishi, Takara, and Kamiyama.

b) Sowing and Cultivation: Sowed on 17 April; transplanted on 24 May; fertilization per 10 a, 40 kg of ammonium sulphate, 33 kg of superphosphate of lime, 10 kg of chlorate of potash (these are primary fertilization), and 11.3 kg of ammonium sulphate (additional fertilization).

c) Inoculation and Investigation Method: 52 strains as shown in Table 46 were selected out of 89 strains listed in Table 45. The breakdown of the sample strains by lyso-types are: Type A...30 strains, Type B...13 strains, Type C...5 strains, Type E...4 strains.

Inoculation: Bacteria, slope cultivated on semi-synthetic agar culture medium at 28°C for 4-5 days, were suspended with sterilized water and made into about a 10^8 /ml density. This was injected with the needle bundles of five sowing needles on 11 and 12 August.⁵⁸ Inoculations were on four pieces each for one strain and one variety, and on 10 leaves for each piece at the upper developed leaves avoiding the central leaf ribs.³¹

Investigation: The diseased areas (mm^2) per four pieces each strain and each variety, eight leaves per each piece were measured on 11 and 12 September.⁵⁷

2) Test Results and Observations

The mean diseased areas of the inoculation tests of each strain by variety (eight inoculated leaves for each piece x four pieces = 32 leaves) are shown in Table 46. And the results of the dispersion analysis of the Table 36 are shown in Table 47.

The author has used two groups of rice plant varieties whose resistance and degrees of resistance have been examined for several years since 1950 by Kiriu.⁴⁹ In this experiment, as is also shown in the dispersion analysis in Table 47, the varieties used for determination are grouped into two groups of strong and weak. In other words, the varieties for determination are roughly classified into the following:

Resistance varieties...Akajinriki, Ogyoku, Norin No.

Table 46

Results of Inoculation Tests--Diseased Area (mm²)

③ 菌番号		② 品種名	④ 黄玉	⑤ 赤神力	⑥ 全防26号	⑦ 地林27号	⑧ 神田1号	⑨ アサカゼ	⑩ ヤチコガネ	⑪ 地林18号	⑫ 宝	⑬ 神山	⑭ 十石
A 型 菌	⑮	H5801	14	11	12	22	21	25	32	69	36	38	110
		H5803	41	32	47	30	42	83	68	67	94	69	93
		H5804	25	16	30	34	32	24	59	99	77	33	146
		H5805	3	5	2	2	6	1	20	39	25	18	188
		H5807	7	2	0	1	1	1	13	30	20	13	45
		H5809	55	33	53	63	24	41	44	77	65	73	107
		H5812	30	12	31	39	30	39	26	80	36	36	124
		H5814	9	4	4	11	7	5	12	17	11	21	29
		H5815	16	10	10	13	15	19	35	32	19	31	45
		H5817	37	31	35	33	33	18	18	32	40	68	62
		H5818	1	0	1	1	1	0	16	13	25	37	30
		H5819	0	0	0	0	0	0	9	18	27	20	21
		H5820	0	0	0	0	0	0	28	17	42	51	22
		H5852	37	27	22	14	18	13	18	37	27	19	33
		H5853	24	26	26	18	23	16	22	31	37	41	49
		H5901	56	27	19	61	43	41	108	88	52	52	146
		H5903	14	18	13	14	10	12	35	23	16	23	38
		H5905	68	63	71	84	73	76	129	118	77	142	158
		H5825	1	1	1	3	1	0	22	27	31	20	50
		H5829	24	30	26	9	15	9	8	24	27	11	17
		H5833	3	2	1	1	1	1	80	85	57	54	172
		H5838	0	1	1	1	1	1	33	36	53	57	80
		H5841	6	4	4	5	5	6	15	14	11	16	39
		H5844	0	0	0	0	0	0	50	19	32	23	41
		H5845	1	0	1	1	1	0	31	23	23	17	44
		H5848	1	1	1	1	0	0	43	19	22	16	25
		H5850 (S)	0	0	1	0	0	1	41	50	14	4	33
		S(新庄菌)	1	1	5	2	2	1	1	1	3	4	3
A' 型 菌	⑯	H5823	3	5	4	6	4	4	36	52	46	48	87
		H5827	1	2	1	2	0	0	19	30	18	37	26
B 型 菌	⑰	H5802	36	29	58	42	56	44	36	50	27	36	112
		H5808	52	44	77	129	125	114	61	72	77	59	102
		H5821	1	2	2	5	3	4	32	27	29	24	111
		H5822	5	4	1	4	2	3	28	35	17	25	81
		H5828	0	0	1	0	0	0	13	16	11	15	31
		H5835	21	18	20	16	20	26	28	29	39	28	66
		H5840	1	0	1	0	0	0	15	12	21	25	28
		H5842	0	0	0	1	0	7	42	39	38	22	60
		H5846	2	3	1	3	1	2	61	59	47	47	86
		H5847	1	1	0	0	0	0	24	46	27	27	56
C 型 菌	⑱	H5849	0	0	0	1	0	0	31	23	20	10	39
		H5851 (B)	0	0	0	0	0	0	8	15	10	11	24
		B(紅粉屋菌)	20	14	39	33	16	12	12	6	6	12	35
		H5810	16	14	20	17	20	11	16	21	19	33	37
		H5826	1	1	3	2	2	2	61	81	47	62	98
E 型 菌	⑲	H5832	3	4	4	3	4	4	15	12	12	12	14
		H5839	1	0	0	1	1	1	4	9	9	7	8
		H5843	0	0	1	1	2	0	46	28	44	37	132
E 型 菌	⑳	H5811	9	15	15	26	8	11	30	36	21	27	39
		H5824	1	3	0	0	2	0	28	26	25	26	55
		H5831	7	7	5	7	5	6	151	151	147	110	300
		H5836	1	0	0	0	1	1	16	16	18	14	40

Legend: 1) Rice variety; 2) Ogyoku; 3) Akajinriki; 4) Zensho No. 26; 5) Norin No. 27; 6) Kamizeki No. 1; 7) Asakaze; 8) Yachikogane; 9) Norin No. 18; 10) Takara; 11) Kamiyama; 12) Toishi; 13) Strain Number; 14) Type; 15) Shinjo strain; 16) Benikonaya strain.

27, Zensho No. 26, Kamizeki No. 1, Asakaze.

Suceptible varieties...Toishi, Takara, Kamiyama, Norin No. 18, Yachikogane.

Table 47

Dispersion Analysis

Factor	Total Square	Freedom	Dispersion	Dispersion Rate	
Total change	623,982	571			
Among varieties	181,648	10	18,165	47.22**	
Among strains	255,749	51	4,819	12.5 **	
Among groups	7,858	4	1,965	5.0 **	
Within Group	Type A	136,409	27	5,052	13.5 **
	Type A'	1,150	1	1,150	3.0
	Type B	56,855	12	4,738	12.3 **
	Type C	6,591	4	1,648	4.3 **
	Type E	36,886	3	12,295	31.9 **
Error	196,584	510	385		

Next, the pathogenicity of each strain is compared according to the diseased area (the mean value of 11 varieties), without relation to the lyso-types, and shows great differences. The results of the dispersion analysis of all strains suggest significant differences among strains. These are classified in accordance with the Tukey's method into three groups beginning with the one with the largest diseased area. The three groups by strains are as follows:

Group I: H5905, H5831

Group II: H5901, H5803, H5809, H5804, H5802, H5812, H5833

Group III: H5817, H5801, H5826, H5805, H5853, H5846, H5835, H5823, H5843, H5852, H5838, H5815, H5821, H5811, H5810, H5903, H5842, H5820, H5822, Benikonaya, H5829, H5847, H5844, H5824, H5825, H5848, H5850, H5845, H5827, H5913, H5814, H5818, H5841, H5849, H5836, H5840, H5828, H5832, H5851, H5839, Shinjo.

However, the above group classification has no relation to lyso-types; when they are compared according to each lyso-type, even among the lyso-types a significant difference of 1% can be observed as in Table 47. Therefore, the mutual difference within each lyso-type was examined. The results showed that there was no difference within lyso-types A, B, and E; only within lyso-type C was a significant difference, at the rate of 5%, observed.

Against this it was simultaneously observed that each lyso-type had significant differences at the rate of 1% as compared to the pathogenicity of its strains. For this reason the degree of pathogenicity by specific lyso-type cannot be validly discussed. For instance, among lyso-type A, there is the H5905 strain with strong pathogenicity and at the same time H5819 or Shinjo strains with extremely weak pathogenicity; and the H5819 strain with rather weaker pathogenicity than the H5826 strain which belongs to lyso-type C.

The results of the classification of the pathogenicity of each strain by lyso-types as tested previously are shown in Table 48. According to Table 48, Lyso-type A and B are composed of Groups I, II, and III, lyso-type E of Groups I and III, and lyso-type C of strains showing the pathogenicity of Group III.

On the basis that strains that mainly invade the affected varieties are regarded as having weak pathogenicity, and strains that invade even resistant varieties as having strong pathogenicity, strains corresponding to each of these were selected from the results as shown in Table 46, and were further classified by lyso-types as shown in Table 49.

Percentages of strains with strong pathogenicity from Table 49 are 46% for Type A, 31% for Type B, 20% for Type C, and 25% for Type E, or in the order of A, B, E, and C.

Table 48

Relationship Between the Degree of Pathogenicity
of Strains and Lyso-types

Pathogenicity	Group I	Group II	Group III	Total
Lyso-type				
Type A	1	6	21	28
Type B	1	1	11	13
Type C	0	0	5	5
Type E	1	0	3	4
Total	3	7	40	50

Note: Group I consists of strains that invade resistant wet rice varieties; Group II, strains that invade susceptible wet rice varieties, but do not invade strongly resistant wet rice varieties; Group III, strains that do not invade susceptible wet rice varieties.

Table 49

Relationship Between the Pathogenicity of Each
Strain and Lyso-type

Classification Lyso-type	Number of Strains that invade even Resistant Varieties	Number of strains that invade mainly affected varieties	Total
Type A	13	15	28
Type B	4	9	13
Type C	1	4	5
Type E	1	3	4
Total	19	31	50

A conclusion from the foregoing is that in the range of test sample strains the classification of lyso-types by phage affinity is not directly connected with the degree of strain pathogenicity.

4. The Serological Difference of Strains Classified by Lyso-types¹⁶⁷

The pathogen of rice bacterial leaf blight was at first named Bacillus oryzae by Bokura in 1909.¹⁰ However, in 1922 Ishiyama⁴⁵ isolated eight groups of pathogens and six groups of associates. The bacteriological examination of these led to the determination of Bacillus oryzae previously so named by Bokura as an associate co-existing in the diseased section with this pathogen. But the pathogen resembled Pseudomonas stewarti E.F.S. of Indian corns; and the primary pathogen was yellowish with pathogenicity and other characteristics clearly different from Bacillus oryzae. Therefore, this was named Pseudomonas oryzae Uyeda et Ishiyama. Recently, the pathogen was classified into the family of Xanthomonas oryzae as established by Dowson,¹⁴ and is generally called Xanthomonas oryzae, as has been mentioned.

As to the serological reaction of this pathogen, Kuwazuka⁶³ produced anti-serum by using an isolate from Aichi Prefecture, and examined the agglutination reaction to the pathogens isolated and cultivated from the affected rice plants in Shizuoka, Hyogo, Ehime, Nagasaki, and Aichi Prefectures. Thus, he proved that each pathogen showed a high degree of agglutination reaction up to a serum dilution of more than 10,000 times, and proposed that all belonged to the same family. However, Fang, et al¹⁹ have reported that there are different species, such as X. hexandra and X. oryzicola that have different serological and biochemical characteristics and with different pathogens.

The author classified X. oryzae into four lyso-types from the affinity relationships of four groups of phages, and examined, as in III, the relation between each lyso-type and pathogenicity. In this experiment, the author further made electron microscope observations of several strains of five kinds of lyso-types in regard to their serological differences and morphologies.

1) Test Method

a) Production of Bacterial Anti-serum--Antigen: Bacterial suspension of about 10^8 /ml (Bacteria slope cultivated on semi-synthetic agar culture medium at 28°C for 20 hours, and a suspended in Ringer-Locke solution** were used).

**Ringer-Locke physiological isotonic sodium chloride solution...1,000 ml of distilled water, 7 gr of sodium chloride, 0.2 gr of KCl, 0.2 gr of CaCl₂, and 0.1 gr of NaHCO₃.

Injection Method: At 4-6 day intervals inoculation was done in the following manner: 1st and 2nd time, 0.5 ml of fume heated sterilized bacteria at 60°C for 10 minutes injected in the abdominal cavity; 3rd time, 0.3 ml intra venously; 4th time, 0.5 ml of bacteria suspension in the abdominal cavity; 5th time, 0.5 ml of the same bacterial suspension intra venously; 6th-9th time, 1-3 ml intra venously. 20 ml of blood was obtained by the heart piercing method; then serum was isolated by the usual method and inactivated; then centrifugally isolated for refining.

b) Test sample strains: The following ten test sample strains were used. Those with * were used as antigen strains at the time of serum production.

Type A...Shinjo,* H5921*, H5801
Type B...Benikonaya*, H5802
Type C...H5839*, H5832
Type D...H5925*
Type E...H5913*, H5824

All these test samples were cultivated in semi-synthetic agar culture media at 28°C, for 20-24 hours.

c) Serological reaction:¹³ This was conducted by agglutination in the test tubes by the dilution method, and the presence of bacterial inactivation was examined by the neutralization method.

d) Effect: Sera were made by the method of stage dilution of two times into 25 times, 50 times, 100 times, 200 times, 400 times, 800 times, and 1,600 times solutions of the primary antisera. The diluted sera solution was poured into small test tubes, 0.5 ml each, and mixed with the live bacterial suspension (produced by suspending bacteria on the Ringer solution diluted 20 times, and with a bacterial density of 10^8 /ml, slope cultivated on the semi-synthetic agar culture medium at 28°C for 20 hours) of each strain to be examined, and was allowed to rest, after shaking, in the thermostat at 30°C for two hours. While comparing this with the natural agglutination examination tube (normal serum mix solution) made for comparison of standards, the \pm of the agglutination was examined. Furthermore, in parallel with this, about 10 ml of bacterial solution of about 10^6 /ml of each strain to be examined was produced. This was mixed with 0.1 ml each of the anti-serum of each strain, and allowed to react for about three hours, twice centrifugally rinsed, mixed with the solution of the semi-synthetic culture medium at 55°C, poured into a bowl for plating, preserved in a thermostat at 28°C, and

then the presence of the bacterial colonies was examined.

e) Electron Microscope Observation: The strains used for this experiment were the same as the ones in the morphological observation as described in Section 3, Chapter VI.

2) Test Results and Observations

The results of the serum cross reaction (the presence of agglutination) are shown in Table 50.

In the inactivation test according to the test method described in d), determination was impossible due to severe contamination during the centrifugal operation.

As shown in Table 50, each antigen strain produced mutual and alternate agglutination reactions according to the anti-serum belonging to each lyso-type. It was proved that the serological difference among lyso-types, that is, the morphological and antigenic characteristics of antigen structures, were the same. As observed in the morphological observation described in Section 3, Chapter VI, the results of electron microscopic photography showed no noticeable differences among lyso-types.

Table 50
Serological Cross Reaction Test by Lyso-types

Lyso-type	Anti-serum Strain to be examined	Type A Serum Shinjo Strain	Type A Serum H5921	Type B Serum Benikonaya Strain	Type C Serum H5839	Type D Serum H5925	Type E Serum H5913
Type A	Shinjo strain	+	+	+	+	+	+
	H5921	+	+	+	+	+	+
	H5801	+	+	+	+	+	+
Type B	Benikonaya	+	+	+	+	+	+
	H5802	+	+	+	+	+	+
Type C	H5839	+	+	+	+	+	+
	H5832	+	+	+	+	+	+
Type D	H5925	+	+	+	+	+	+
Type E	H5824	+	+	+	+	+	+
	H5913	+	+	+	+	+	+

Note: By the 800 times dilution tube, + and - of agglutination was determined.

5. Summary

1) Xanthomonas oryzae (Uyeda et Ishiyama) Dowson, the pathogen of rice bacterial leaf blight, is classified by the host range of four groups of phages, into five lyso-types (A, B, C, D, and E). Their relationships are briefly shown in Figure 7.

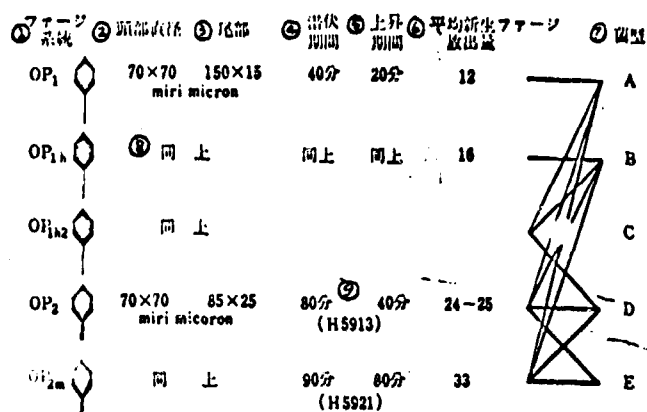


Fig. 7 Relationship Between the Pathogenic Phages of Rice Bacterial Leaf Blight and Lyso-types

[Legend]: 1) Phages; 2) Head diameter; 3) Tail; 4) Latent time; 5) Rise time; 6) Average burst of new phages; 7) Lyso-type; 8) Same; 9) Minutes.

2) Lyso-type does not constitute the criteria for classification of the degree of pathogenicity. However, it seems that type C strains include strains of weak pathogenicity.

3) No serological difference among lyso-types has been observed.

4) Morphological observation with the electron microscope does not reveal strain differences.

5) It is noteworthy that there are many strains that invade such resistant varieties as Ogyoku, Zensho No. 26, and Norin No. 27.

Section 3. The Quantitative Test of the Pathogen of Rice Bacterial Leaf Blight by OP₂ Phages

The methods of examining for the presence of and the quantitative method for the pathogen of rice bacterial leaf blight were devised by Wakimoto, and Yoshii,^{135,138} A simplified method was devised by Wakimoto.¹⁴² This quantitative method was widely used thereafter for ecological study of rice bacterial leaf blight, leading to the discovery of many new facts. The test method in this experiment also roughly follows this. However, the details of this quantitative operation are very complicated, as described in Chapter VII, and require skill.

The author pursued the shifts in bacterial multiplication in the fields in his research into the ecology of rice bacterial leaf blight, using this method. In executing the test, the time needed for phage adsorption by the bacteria to be measured and for completing various quantitative operation is limited to 45 minutes, or the latent period of OP₁. Consequently, the experiment is very busy. In contrast to this, in the aspect of the ecological study of the multiplied bacteria in the fields, because of the time limit of the latent period in the complicated phage quantitative method, the test samples to be measured at first are limited naturally to four to six samples. But OP₂, isolated by the author, as shown in 7. Section 1, has a latent period of 70-80 minutes, or about twice that of OP₁. This characteristic allows for the simultaneous quantitative treatment of a maximum of 12 samples the first time with the rotor hole of the centrifugal machine. Also, OP₂ has affinity with A, B, D, and E types; its host range is the widest among the known phages; and the burst of new phages of OP₂ is over 20, more than 12-16 more than OP₁ and OP_{1h}. Therefore, it can be theoretically acknowledged that OP₂ is advantageous even for samples with a small volume of bacteria to be measured.

1. Quantitative Comparison of Bacteria by OP₁ and OP₂

1) Experimental Method

Quantitative method is shown in Figure 8. In this experiment, the number of bacteria to be measured was prepared in the range of $n \times 10^3$, and the quantitative limits of OP₁ and OP₂ were examined in concentration stages. Shinjo and H5801 of type A with affinity with both OP₁ and OP₂ were selected for measurement.

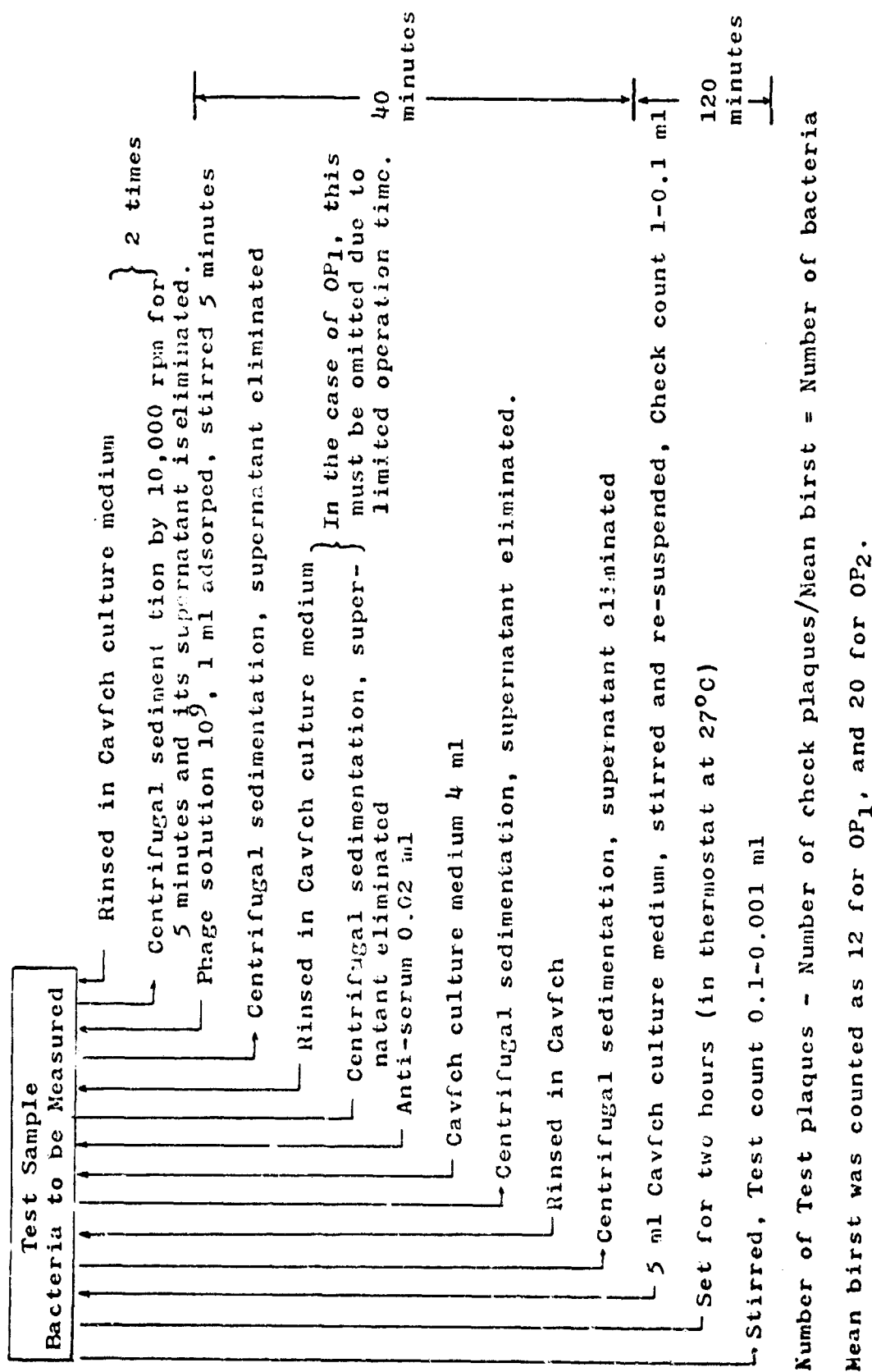


Fig. 8 Quantitative Method of Bacteria by OP₁ and OP₂

2) Experimental Results and Observations

The measurement of the bacteria using OP₁ and OP₂ according to the method shown in Figure 8 is shown in Table 51. The number of bacteria in the test samples was checked by the culture colony on the semi-synthetic culture plate.

Table 51

Comparison of Quantitative Test of
Bacteria by OP₁ and OP₂

② ① 定量的に 供した ファージ	n				n × 10 ²				n × 10 ³				n × 10 ⁴				n × 10 ⁵			
	I	II	III	平均	I	II	III	平均	I	II	III	平均	I	II	III	平均	I	II	III	平均
OP ₁	0	0	0	0	0	0	0	0	0	0	0	0	8	5	22	12	377	249	1,080	569
OP ₂	0	0	0	0	0	0	1	0.3	11	7	33	17	166	99	3,600	1,288	8,870	58,960	3,130	23,655

Legend: 1) Number of bacteria in sample;
2) Phage used for quantitative test; 3)
Average.

As shown in Table 51, while the quantitative test is difficult when the number of bacteria in the sample of OP₁ phage is more than 10³, in the case of OP₂, the quantitative test is possible even when the bacterial concentration is at 10². However, in either case, estimation does not match the rough number of bacteria in samples; and the fact that estimation was less than 1/10 of the measured value of the actual number, seems to indicate the limits of the phage quantitative method.

2. On the Nonjoining Phenomenon of the OP₂ Strain

The method of calculating plaques is an important part in the quantitative bacteria by phage method. Estimation is to be made when the multi-layers of raw agar are made into plaques, but in many cases the raw agar layers are omitted. Generally the plaques of OP₁ group are joined together in about ten hours, which makes the counting of plaques difficult. On the other hand, in the case of OP₂, such a phenomenon of joining of plaques does not occur, and counting is easy.

3. Summary

The quantitative method of the bacteria by OP_2 seems to have wide and good applicability, due to its wide host range, the latent period from adsorption to the first of new phage particles is long, and its mean volume of first is large, and there is no joining of plaques.

CHAPTER VII. EXPERIMENTAL METHODS ON THE QUANTITATIVE TEST OF THE PATHOGENS OF RICE BACTERIAL LEAF BLIGHT¹³⁸

In the experiments concerning the over-wintering of the pathogen of rice bacterial leaf blight and its primary and secondary infections, the detection and cycle of the pathogens were examined. In this, the quantitative test of the pathogens covered the major portion of the experimental operation. For this reason, experimental methods will be explained in detail.

Section 1. Test Samples

In the case of irrigation water, it was scooped into pre-arranged sterilized containers and certain volumes were used as test samples. Rice plant leaves and other plant materials were placed in 500-1,000 ml jars with large mouths, to which proper volumes of sterilized water were added, shaken, and rinsed. The bacterial suspension was used for samples. The sample volume was prepared according to the estimates of existing bacteria.

Section 2. Experimental Instruments

All the experimental instruments were sterilized. Test tubes, rubber lids, squirts, and other small instruments were sterilized by boiling before use. For collecting rice leaves, pincers sterilized with 70% alcohol or fingers were used, and during the experiment particular attention was paid to keeping finger tips sanitary.

Section 3. Quantitative Method of Bacteria

1. Quantitative Phage Test

1) Operation: As has been stated, the pathogen of rice bacterial leaf blight by the phage quantitative method (hereafter this will be referred to simply as the phage

method) was devised by Wakimoto and Yoshii.¹³⁸ In this method, when phage particles with affinity to bacteria of particular lyso-types is adsorbed by bacteria, they destroy bacteria after a certain period (the latent period), and the phage particles which multiplied in bacteria are released. (From the rise period to burst). In this case, when the environmental conditions (the nutrition and temperature of bacteria) that directly affect the multiplication of phages are kept constant, the relationship between the particular phages and particular bacteria and the average number of phage particles burst from each stage and time of phage multiplication from bacteria is always constant. The phage method is to count according to the plaque formation method the bacterial volume in rice plant leaves, irrigation water, and in soil that contain the pathogen of rice bacterial leaf blight.

The present experiment essentially followed the Wakimoto and Yoshii method, but some modifications were made. This is briefly shown in Figure 9, 1-4.

Method 1

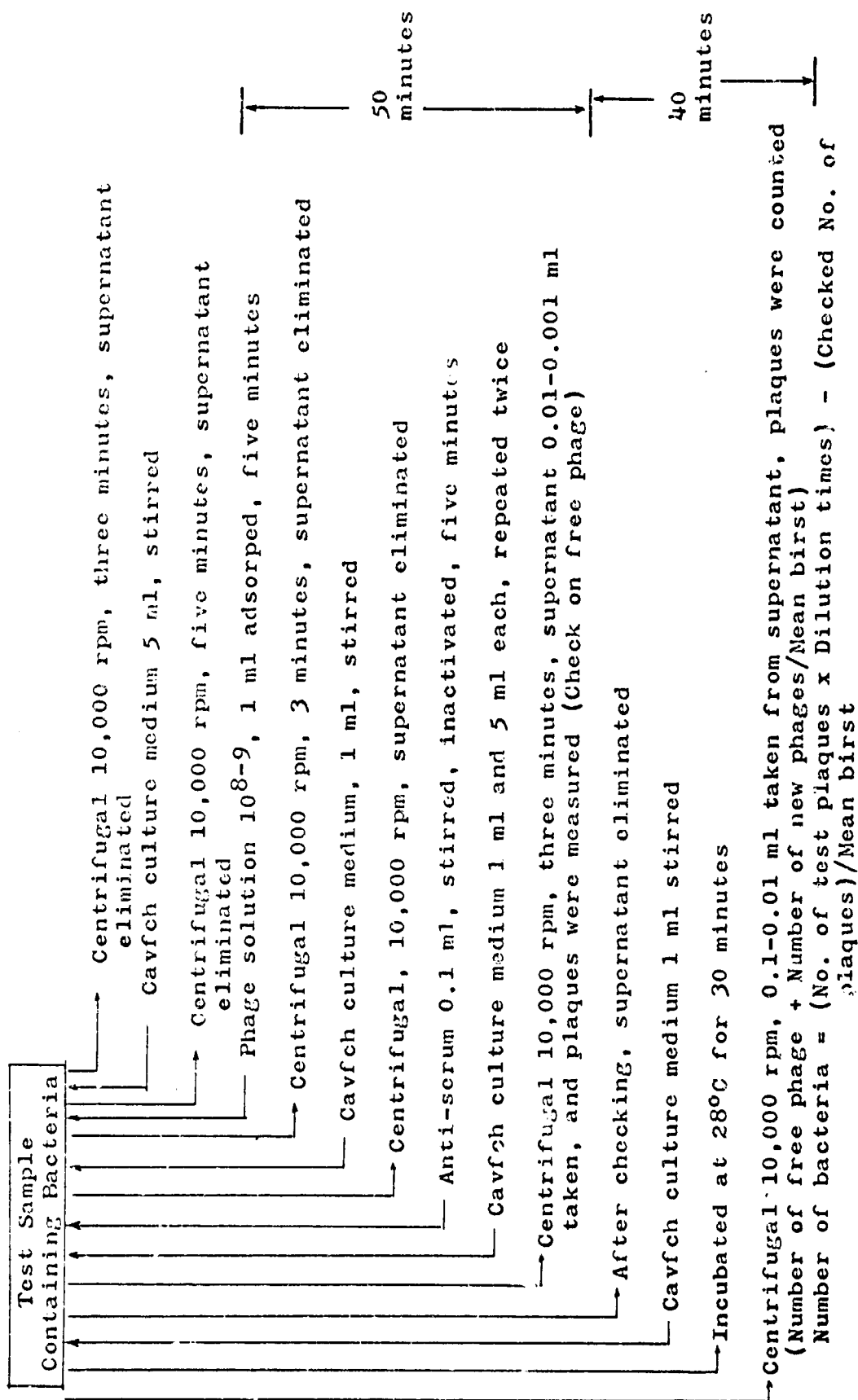
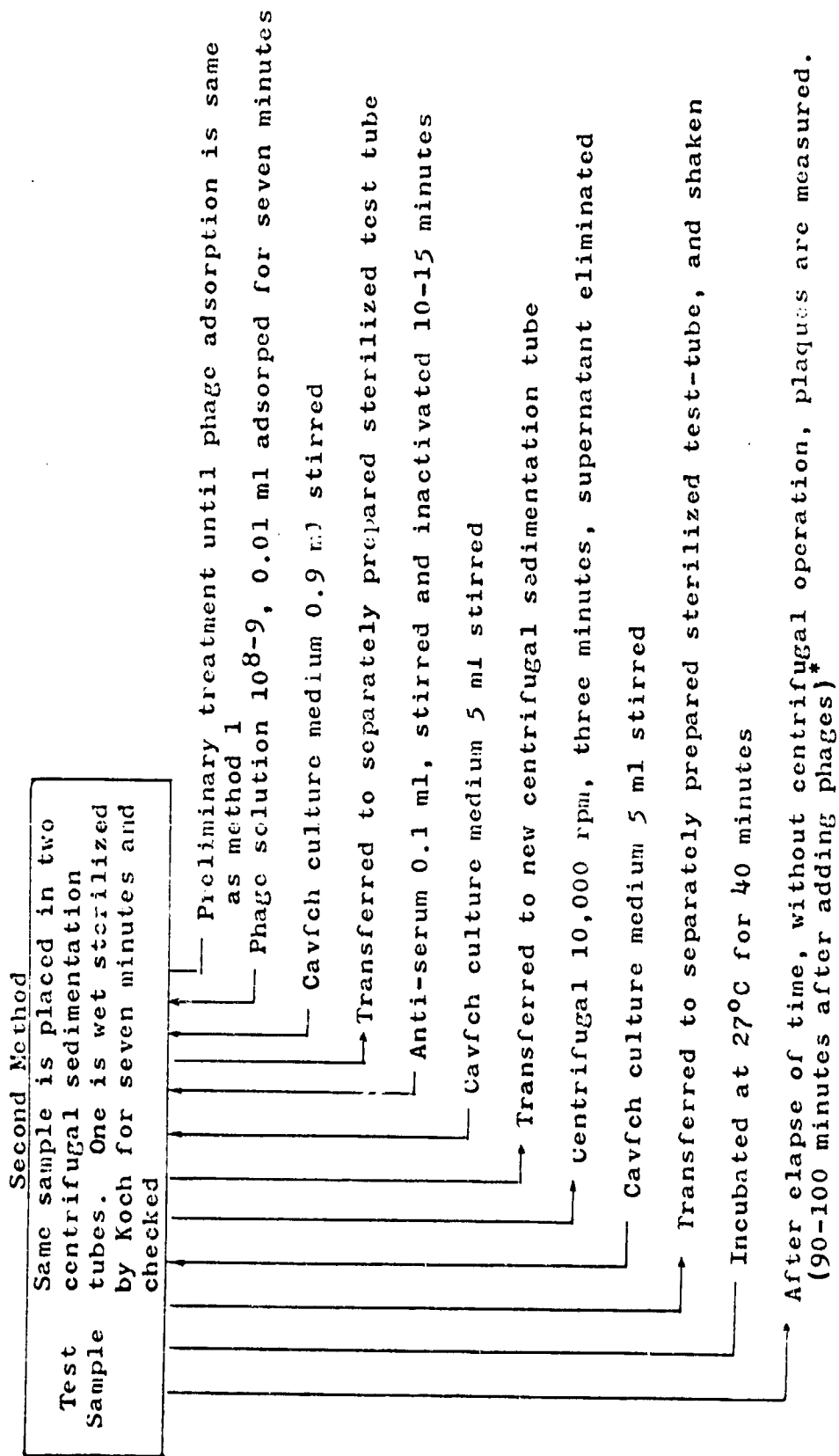


Fig. 9-1 The Quantitative Method of the Pathogen of Rice Bacterial Leaf Blight by Bacteriophage (Wakimoto and Yoshii)



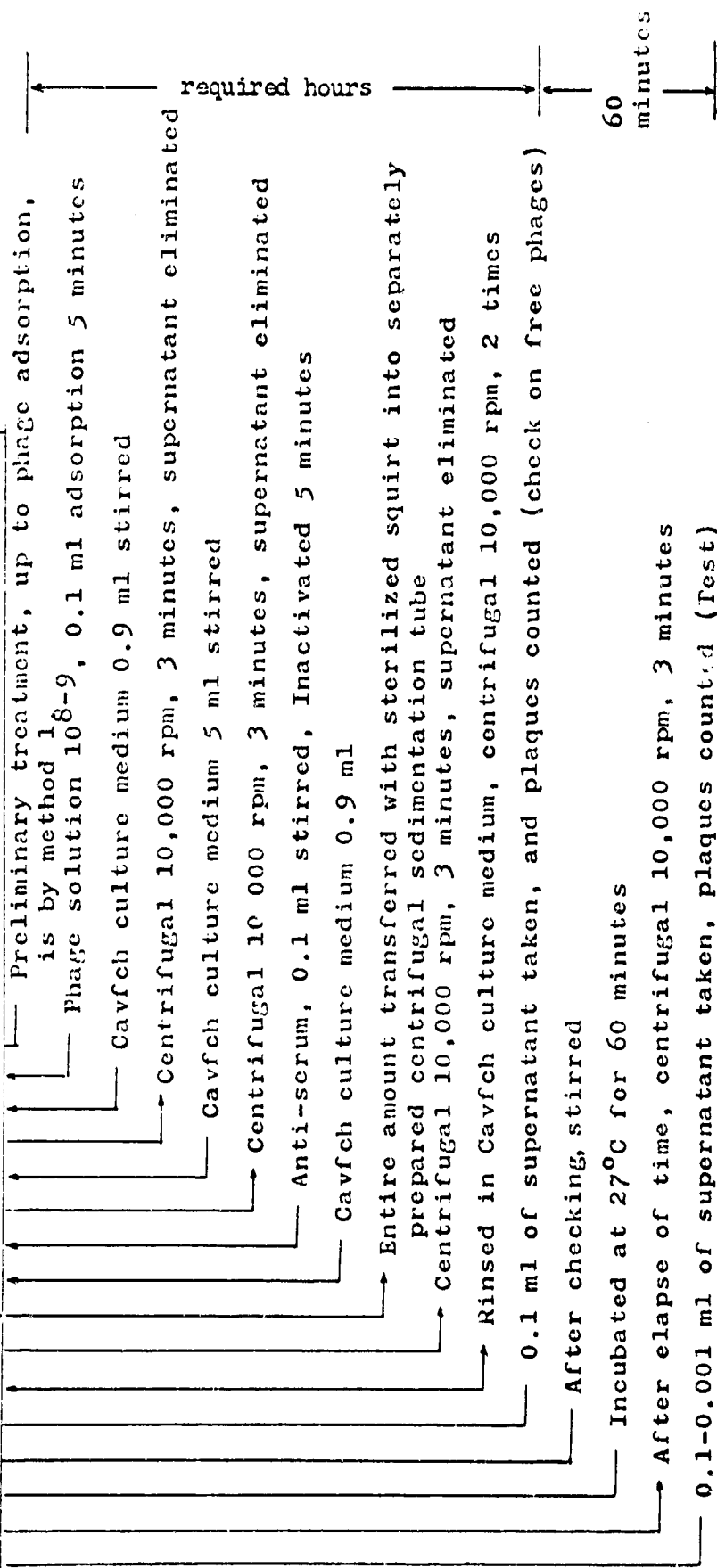
*Number of bacteria is counted from the number of plaques in test sample and check sample.

Number of Bacteria = (Number of plaques in live sample x Dilution rate)
 - (Number of plaques in sterilized sample)/Mean first

Fig. 9-2 The Quantitative Method of the Pathogen of Rice Bacterial Leaf Blight by Bacteriophage (Wakimoto and Yoshi)

Third Method

Test Sample Same sample is placed into two centrifugal sedimentation tubes, making them an identical pair during operation. This allows for the balancing operation before centrifugal, curtails time, and allows simultaneous repeated tests.



*When OP₁ is used for adsorption ...50 minutes,
 " " " " ...75 minutes.

No. of Bacteria = (No. of Test plaques x Dilution rate) - (No. of check plaques)/Mean first

Fig. 9-3 The Quantitative Method of the Pathogen of Rice Bacterial Leaf Blight by Bacteriophage (Wakimoto and Yoshii)

Fourth Method

Test	Sample is ground and diffused in Cavfch culture medium, bringing the total volume to 50-100 ml
------	--

Phage solution* $\times 10^4-5$... this is accurately checked beforehand by plaque counting

Sterilized rubber lid is tightly placed, shaken, cultured for 3-4 hours

Centrifugal 6,000 rpm, supernatant is diluted 10-1,000 times, and number of plaques is tested for.

*Diffused and suspended on Cavfch culture medium.

Number of Bacteria = $\frac{(\text{Number of Test plaques} \times \text{Dilution rate}) - (\text{Number of check plaques})}{\text{Mean burst}}$

Fig. 9-4 The Quantitative Method of the Pathogen of Rice Bacterial Leaf Blight by Bacteriophage (Qakimoto and Yoshii)

2) The Composition of the Medium used for the Quantitative Test

The composition of the Cavfch (Vitamin free casein-hydrolysisate calcium medium^{134,143}) used in the phage method is shown in Table 52.

Table 52

Composition of Cavfch Medium

CaCl ₂	0.5 gr
Vitamin free casein-hydrolysisate, 10% liquid	10.0 ml
Cane Sugar	10.0 ml
Distilled Water	1,000 ml

The composition of the semi-synthetic agar culture medium used for the plaque count method is shown in Table 10.

3) Phages and Strains used for the Quantitative Test

a) Phages: OP₁ and OP₂.

b) Strains: K strains and Shinjo strains in the possession of the Kyushu District Agricultural Experimental Station, H5801 (the foregoing are all Type A) and H5913 strain (Type E) in the possession of the Hokuriku Agricultural Experimental Station.

4) The Preparation of the Phage Solution

To 2 ml of concentrated bacterial suspension with an affinity with OP₁ and OP₂, 10 ml of the semi-synthetic agar culture medium dissolved at 50°C was added and mixed. This was allowed to flow into a bowl to be made into plates, and then the OP₁ or OP₂, isolated beforehand, was transplanted by needles to several points on the plate. This was preserved in a thermostat at 28°C for 24 hours to produce bacteria. Then the agar with bacteria was cut into 1 cm² pieces, and then transferred to 10 ml of the Cavfch culture medium. By shaking this several times, about 10⁸⁻⁹/ml of the primary solution of the titre of each phage was produced. The bacteria and bacterial pieces in this were precipitated with a centrifugal machine, and the supernatant solution was transferred to a separate test tube. This was then allowed to set for about an hour in a thermostat at 28°C for the quantitative test.

5) Anti-serum

Using the concentrated phage produced by the method described in 4) as the antigen, this was injected into house rabbits (adult rabbits, about 4 kg in weight) and anti-serum was produced. They were injected on more than ten occasions every three or four days. For the first to fourth times, the inoculation was made into the abdominal cavity, and for the fifth through 12th times it was made in the veins of the ear. The dosages were 0.1 ml the first time, 0.5 ml the second time, 1 ml the third time, 0.1 ml the fourth time, 0.5 ml the fifth time, 1 ml the sixth time, and 2-3 ml thereafter. In this way, inoculation was continued for about 45 days, and blood was taken from the veins in the ear. After confirming its high factor, about one week after the last day of inoculation, 3 ml of phage was injected twice at four day intervals. After one day's fast, blood was taken. The blood was preserved in a beaker at 37°C. After it coagulated, it was stripped off the wall of the tube, and left in an ice box for 24-48 hours,

thereby isolating serum. The serum thus produced was isolated by a centrifugal machine at 10,000 rpm for 10 minutes, and the supernatant serum was heated and inactivated in hot water at 56°C. This was either frozen as it was or frozen and dehydrated for prolonged preservation.

Furthermore, for the quantitative test or other experiments on phages, the antibodies of bacteria were sufficiently aggregated and neutralized beforehand to eliminate its inactivation capacity against bacteria. Experiments on phages require high speed centrifugal sedimentation operation. For this reason, in this experiment the H-200 type Kokusansha or YK-B-3 type Kubota Centrifugal machine with maximums of 16,000 rpm were used.

2. Quantitative Method with Streptomycin Resistant Bacteria

The quantitative test method of the volume cycle of bacteria using streptomycin resistant bacteria as tracers was devised by the First Bacteria Research Section of the Agricultural Technology Research Center.^{85,117} This method is applied for the quantitative test of bacteria in soil, which are difficult test samples to estimate by the phage method (inoculation test).

1) The Composition of Resistant Bacteria and Isolation Medium

The SR-2 strain donated by the First Bacterial Research Section, Department of Pathology, Agricultural Technology Research Center, was used as the test sample, and for the isolation medium the improved potato ring rot medium as shown in Table 53 was used.

2) Operation

A certain volume of the test sample was mixed with a proper amount of water. After shaking well this was allowed to stand for an hour, and its supernatant was transferred to the sterilized test tube. Then the medium prepared separately, as in Table 53, was dissolved into this. Then this was allowed to flow quickly into a bowl and made into plates. These were cultured for 3-4 days in the thermostat at 27°C. Then the number of colonies of streptomycine resistant bacteria formed on plates was measured by the use of a colony computer.

Table 53

Composition of the Isolation Medium for
Streptomycin Resistant Bacteria

Potato	300.0	gr
NaNO ₃	1.0	"
Na ₂ HPO ₄	2.0	"
NaCl	2.0	"
Cane Sugar	20.0	"
Peptone	5.0	"
Yeast extract	5.0	"
Sulphuric Acid Dihydro Streptomycin	1.0	"
Crystal violet (0.09-0.1% sol.)	5.0	ml
Eurocidin	0.1	gr
BTB	a little	
Agar	25.0	gr
Distilled Water	1,000	ml

CHAPTER VIII. THE LIFE CYCLE OF THE PATHOGEN OF RICE BACTERIAL LEAF BLIGHT DURING THE PERIOD OF OVERWINTERING

During the occurrence and development of rice bacterial leaf blight, bacteria are widely distributed in rice plants and in the vicinity of rice paddies. As harvest time approaches, bacterial multiplication declines because of the decrease in temperature and other environmental conditions. Thus density falls. When rice is harvested, some bacteria invade affected rice plants and multiply there, and others are carried away from the rice paddies. Some others remain in cut culms, on the surface soil of rice paddies, on ridges, or in irrigation water. This pathogen lives on other grasses in addition to rice plants. There are many naturally occurring colonies in Sayanukakusa (sic). From this, the pathogen overwinters in the harvested rice plants, on the perimeter of rice paddies, and in host grasses. The author, from this viewpoint, conducted experiments on the life cycle of the pathogen during the overwintering period.

Section 1. The Viability of the Pathogen in Soil¹⁷¹

During a 3 month winter period from December 1959 to March 1960, and during a summer period from July to September 1960, the viability of the pathogen was examined by using the soil from the rice paddies at the Hokuriku District Agricultural Experiment Station.

1. Viability of Cultured Bacteria

1) Experiment Method

By using *X. oryzae* H5801 slope cultured on the semi-synthetic agar culture medium at 28°C for five days, 50 ml of concentrated suspension solution of bacteria ($\approx 10^8$ /ml) was produced. This was mixed with 100 gr each of sterile paddy soil separately air dried and sieved (1 atmospheric pressure by autoclave for 15 minutes), and of unsterilized soil. Then this was placed in a 500 ml capacity flask and closed with cotton, and left in a thermostat at 28°C or outdoors. Then on the dates shown in Tables 54 and 55, the

Table 54

Viability of Cultured Bacteria in Soil
(in fields during winter)

Date of Investigation Area of Experiment	1959			1960		
	18 Dec.	29 Dec.	14 Jan.	7 Feb.	27 Feb.	15 March 28 March
Sterilized Soil + Bacterial So- lution	13,630 58,190	8,850 5,470	73 7,153	1,160 3,010	10 0	0 10 0
Unsterilized Soil + Bacterial Solution	2,033 9,274	3,510 17,490	11,276 11,330	6,280 1,090	0 996	0 0 0
Sterilized Soil + Sterilized Water	0 0	0 0	0 0	0 0	0 0	0 0 0
Unsterilized Soil + Sterilized Water	0 0	0 0	0 0	0 0	0 0	0 0 0

Table 55

Viability of Cultured Bacteria in Soil
(in Thermostat at 27°C)

Date of Investigation Area of Experiment	1959		1960			
	18 Dec.	29 Dec.	14 Jan.	7 Feb.	15 March	28 March
Sterilized Soil + Bacterial Solution	9,346	4,510	0	0	0	0
	11,610	5,160	0	0	0	0
Unsterilized Soil + Bacterial Solution	15,920	3,700	0	0	0	0
	6,370	1,170	0	0	0	0
Sterilized Soil + Sterilized Water	0	0	0	0	0	0
	0	0	0	0	0	0
Unsterilized Soil + Sterilized Water	0	0	0	0	0	0
	0	0	0	0	0	0

Table 56

Temperature During Experiment (By Hokuriku
District Agricultural Experimental Station)

Month-year	Dec. 1959	Jan. 1960	Feb. 1960	March 1960
Temperature				
Higest Tem- perature	5.1 °C	5.3 °C	7.1°C	10.9 °C
Lowest Temperature	0.4	-0.4	-1.2	1.9
Mean	3.3	-2.2	3.0	5.9

Table 57

Viability of Cultured Bacteria in Soil
(in fields during summer)

Area of Experiment	1960						
	24 July	25 July	27 July	29 July	2 Aug.	7 Aug.	11 Aug.
Sterilized Soil + Bacterial Solution	1,207 325	660 4,040	48 2	10 6	0 0	0 0	0 0
Unsterilized Soil + Bacterial Solution	696 9,521	30 216	56 9	6 0	0 0	0 0	0 0
Sterilized Soil + Sterilized Water	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Unsterilized Soil + Sterilized Water	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Mean Temperature during five day period (°C)	26.9		26.3		28.6	28.2	24.0

Table 58

Viability of Cultured Bacteria in Soil
(in refrigerator at 5°C)

Area of Experiment	1960							
	24 July	25 July	27 July	29 July	2 Aug.	7 Aug.	11 Aug.	19 Aug.
Sterilized Soil + Bacterial Solution	298 852	671 125	942 365	82 36	51 4	8 1	10 10	7 3
Unsterilized Soil + Bacterial Solution	307 1,761	3,570 403	121 575	390 297	111 64	4 4	0 0	1 3
Sterilized Soil + Sterilized Water	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Unsterilized Soil + Sterilized Water	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

Note: Detection tests were made on 23 and 29 August with negative results. (0)

viability of bacteria was examined in each kind of soil by the phage quantitative method. As a comparison a bacteria free area (50 ml of sterile water added to sieved soil) was established as the check against each quantitative test. For the quantitative test of bacteria each time, two flasks were used. 100 ml of sterile water was added and soil was mixed with glass rod and let rest for three hours. Its supernatant solution was separated and centrifuged at 2,000 rpm for two minutes in order to eliminate foreign matters and soil grains. Then its supernatant solution was diluted by ten times at the Cavfch culture medium and used this as the primary test sample solution. The phage quantitative method followed the third method in Figure 9, and 5-20 ml of the primary test sample solution was tested.

2) Experimental Results and Observations

The results of experiments on the viability of cultured bacteria in soil are shown in Tables 54-58.

As shown in Tables 54 and 57, bacteria survived in soil for about 50 days from 18 December to 7 February with on outside temperature averaging 3°C during winter, and about five days under outdoor conditions in summer from 24 to 29 July. The period of viability during summer is much shorter than during winter. This generally agrees with the results of experiments in preserving bacteria in a thermostat at 28°C and in a refrigerator at 5°C as shown in Tables 56 and 58. Viability for the duration of 11 days at 28°C and of 26 days at 5°C was observed. That is, the period of viability of the pathogen of rice bacterial leaf blight in soil showed considerable fluctuation according to temperature.

2. The Viability of Streptomycin Resistant Bacteria

1) Test Method

About 10^8 /ml of concentrated suspension of streptomycin resistant SR-2 strain slope cultured for six days on a semi-synthetic culture medium with the addition of 0.15% streptomycin was mixed with 100 ml in flasks containing 200 gr each of the sterilized soil of the rice paddies of the Hokuriku District Agricultural Experimental Station (pH 4.8) prepared beforehand, air-dried, and strained and unsterilized soil. During the periods shown in Tables 57-59 the presence of bacteria and their volumes were examined.

As a comparison, an area of no streptomycin resistant bacteria (sterilized water in equal volume to the bacterial solution was added) was prepared and used as a check

for each time. For the bacteria quantitative test, the aforementioned two flasks were used. 200 ml of sterilized water was added to this, and after stirring with a glass rod this was allowed to stand for two hours. The supernatant was made into a 100 times solutions (dilution 1), 10^{-3} solution (dilution 2), and 10^{-4} solution (dilution 3) with the Cavfch culture medium which had 0.1% glutamic acid added. One ml each was taken from this diluted solution, put into separate test tubes, and mixed with 10 ml of the agar culture for the isolation of streptomycin resistant bacteria dissolved at 55°C . This was immediately poured into a bowl, and flat cultured at 26°C . In this way, it was cultured for 5-7 days, taken out of the thermostat, and the number of colonies of streptomycin resistant bacteria thus cultured and formed was counted.

2) Experimental Results and Observations

The foregoing experimental results are shown in Table 59. The viability of streptomycin resistant bacteria in the thermostat adjusted at $19-22^{\circ}\text{C}$ is shown in Table 60, and viability in a refrigerator at $0-5^{\circ}\text{C}$ is shown in Table 61, respectively.

Table 59

Viability of Streptomycin Resistant Bacteria
in Soil (at room temperature; 1960)

試 験 区	②7月6日③			7月11日		7月26日	
	稀釈②-1	稀釈-2	稀釈-3	稀釈-1	稀釈-2	稀釈-0	稀釈-1
殺菌土塊⑤	2,212	524	10	5	0	0	—
+ 菌液⑥	5,572	62	4	7	0	0	—
無殺菌土塊⑦	876	193	12	0	0	0	—
+ 菌液⑧	9,190	987	77	18	0	0	—
殺菌土塊⑨	0	0	0	0	0	0	—
+ 菌液⑩	0	0	0	0	0	0	—
無殺菌土塊	0	0	0	0	0	0	—
+ 菌液	0	0	0	0	0	0	—

Note: *After left in a room, the mean temperature at 10 a.m. was 27°C . Figures in upper columns stand for the first experimental results, and lower columns for the second ones. The same is true of Tables 60, and 61.

/Legend/: 1) Area of test; 2) Month; 3) Day; 4) Dilution; 5) Sterilized soil; 6) Bacterial solution; 7) Unsterilized soil; 8) Sterilized water.

Table 60

Viability of Streptomycin Resistant Bacteria in Soil (in a thermostat at 19-22°C, 1960)

試 驗 区	② 8月5日	③ 8月7日	8月18日	8月30日	9月12日	10月1日	10月15日	11月7日
	稀釈④ 3	稀釈—3	稀釈—1	稀釈—1	稀釈—1	稀釈—1*	稀釈—0*	稀釈—0*
殺菌土壌液⑤	872	112	29	0	0	0	0	0
+ 菌液⑥	180	301	24	0	0	0	0	0
無殺菌土壌液⑦	995	692	83	0	0	0	0	0
+ 菌液	75	528	86	0	0	0	0	0
殺菌土水⑧	0	0	0	0	0	0	0	0
+ 菌水	0	0	0	0	0	0	0	0
無殺菌土水	0	0	0	0	0	0	0	0
+ 菌水	0	0	0	0	0	0	0	0

Note: *Measurement was difficult due to miscellaneous bacteria (less miscellaneous bacteria only in the unsterilized soil + Bacterial solution area).

/Legend/: 1) Area of test; 2) Month; 3) Day; 4) Dilution; 5) Sterilized soil; 6) Bacterial solution; 7) Unsterilized soil; 8) Sterilized water.

Table 61

Viability of Streptomycin Resistant Bacteria
in Soil (in a refrigerator at 0-5°C)

①	8月5日	8月7日	8月17日	8月28日	9月12日	10月15日	10月24日	11月2日	11月10日	11月17日	11月30日	12月2日	12月16日
試驗區	稀積 -3	稀積 -2	稀積 -1	稀積 -1	稀積 -1	稀積 -0	稀積 -0	稀積 -0	稀積 -0	稀積 -0	稀積 -0	稀積 -0	稀積 -0
殺菌土培 + 菌液	872 1,680	1,162 990	1,550 552	109 57	4 7	0 63	85 0	17 15	29 24	0 4	5 5	2 5	1 0
無殺菌土培 + 菌液	995 75	104 524	62 101	43 22	2 23	66 0	71 22	36 25	38 7	5 2	6 10	6 0	1 0
殺菌土培 + 殺菌水	0	0	0	0	以後 中止								
無殺菌土培 + 殺菌水	0	0	0	0	以後 中止								

Note: After two weeks the number of bacteria showed drastic decreases.

Legend: 1) Area of test; 2) Month; 3) Day; 4) Dilution; 5) Sterilized soil; 6) Bacterial solution; 7) Unsterilized soil; 8) Sterilized water; 9) Suspended hereafter.

As shown in Tables 59-61, the viability of streptomycin resistant bacteria varies with temperatural environment. It is five days at a room temperature at 27°C, 13 days at 19-22°C, and 38 days at the low temperatures of 0-5°C. Especially in the experiments at 5°C, detection was made, if of only a slight degree, in the period from 5 August to 16 December. This is 133 days in total. This is a question to be examined, because the viability indicated here is rather long despite the strained soil containing little organic matter and under an environment where no live plant roots existed. Throughout this experiment the relationship between viability and sterilization of soil was not clarified.

3. Viability of Dried Bacterial Ooze

In the affected leaf of rice bacterial leaf blight, the bacteria multiplied in the tissues, secrete out from the water holes along the leaf edge or disease scars to form ooze (See photograph 5). This ooze either adheres to the leaf after being dried and hardened, or drops into rice paddies through the impact of wind, etc. When the ooze is hardened, it does not easily dissolve or diffuse in water.

Also it is usual that ooze drops to the surface soil of rice paddies without water, and survives as a concentrated bacterial mass.

In the following, the viability of ooze in wet soil was examined by the needle inoculation method.

1) Experimental Method

Dried bacterial ooze collected in August 1959 from the diseased leaf (variety: Towada) in the affected rice paddies of the Hokuriku District Agricultural Experimental Station was used as test samples. In the test area, 120 grains of dried bacterial ooze was mixed with 10 gr of dried sieved soil (unsterilized). This was divided into fixed glass jars with 25 ml capacities. One half of these were left dried, and the other half were moistened by adding 5 ml of tap water. These jars were closed tightly and left in a room. As shown in Table 62, in each period, the contents were extracted with a spoon and made into an inoculated bacterial solution by adding a small volume of water. Inoculation was made with multiple needles (five needle-bundle) into rice plants, sowed and raised each month. The rice variety used for the inoculation test was Jugoku. The growth phases of the rice plants at the time of inoculation varied, but the rice plants were generally in the early period of root splitting or the period of maximum root splitting. All were cultivated at room temperature. As a comparison, the bacterial solution produced by adding a small amount of water to the dried bacterial ooze preserved in a desiccator (concentration of bacteria was estimated to be about $10^7\text{-}8/\text{ml}$) was needle inoculated in each inoculation period as in the case of the aforementioned mixed soil areas. After inoculation, the rice plants were covered with a vinyl covering for two days, and they were opened so that the diseases could develop. The occurrence of disease was examined between 17-22 days after inoculation, and by the presence of occurrence, bacterial viability was determined. The inoculation test was conducted in the period from October 1959 to April 1960 by using the rice plants cultivated in green houses.

2) Experimental Results and Observations

The results of the examination of the viability of dried bacterial ooze in soil are shown in Table 62.

As shown in Table 62, dried bacterial ooze in soil survived until the following April without losing its pathogenicity. Even in wet soil, it could survive in a glass

Table 62

Viability of Dried Bacterial Ooze in Soil

Date of Inoculation	1959					1960			
	12 Oct.	8 Nov.	21 Dec.	2 Feb.	22 Feb.	19 Mar.	13 April		
Dried bacterial ooze + air dried sieved soil (dried)	16/20 (80)	10/20 (50)	19/21 (90)	7/16 (44)	16/19 (84)	18/19 (53)	8/22 (34)		
Same (wet)	18/20 (90)	9/19 (47)	0/20 (0)	8/21 (38)	10/20 (50)	2/20 (10)	2/19 (10)		
Dried bacterial ooze** (in desicator)	19/20 (95)	21/21 (100)	7/19 (38)	16/21 (76)	14/20 (70)	13/22 (60)	18/21 (95)		

Note: * 29 days after the treatment of dried bacterial ooze in soil.

**One, in a desicator tightly closed under refrigeration, shows pathogenicity even after two years.

Figures as denominators show the number of inoculated leaves, and figures as numerators show the number of affected leaves. Figures in () show the percentages of occurrence.

container for about six months until the following April as did the former. In contrast to this dried bacterial ooze preserved in a desicator had a very high degree of activity until the following April, and it was found, through these tests, that, as noted in Table 62, under good conditions of preservation it could survive two years. Generally large amounts of dried bacterial ooze adhere to affected rice straws. Therefore, it is estimated that rice straws stored indoors can serve as a base for the overwintering of highly concentrated bacteria.

4. Electron Microscopic Observation of Dried Bacterial Ooze

The bacterial suspension produced by dissolving dried ooze three days after its secretion by adding a very small volume of water was further diluted and mesh-mounted. This was dried at room temperature and observed with an Akashi TRS-50 type electron microscope (accelerated voltage 50 KV) and photographed. The results showed that the measurement of bacteria in the dried bacterial ooze was $0.51 \times 0.77 \mu$ (100 pieces were measured), as described in Section 3, Chapter VI, or smaller than the cultured bacteria. But it was observed that there were mixed bacterial cells with high and low electron densities. This seemed to show the change of bacterial cells into durable structures, under dried conditions. (See photograph 32)

5. Summary

The viability of the pathogen of rice bacterial leaf blight varies considerably according to temperature conditions. It was 26 days at 5°C (but the bacteria survived 133 days in the streptomycin resistant bacteria ?), 13 days at $19-22^{\circ}\text{C}$, 5-11 days at $27-28^{\circ}\text{C}$. The foregoing are all the results of the tests in glass containers. Dried bacterial oozes that formed on affected leaves show strong viability, and usually survive until the following April even in wet soil with a little lower rate of survival than in dry soil. If the conditions of preservation of the ooze are good, it can survive for a long period (at least two years), with a very high value as the primary infection source.

Section 2. The Viability of Bacteria in Irrigation Water

1. Viability of Bacteria in Irrigation Water During Winter

1) Experimental Method

Water was taken from the reservoir used for irrigation at the Hokuriku District Agricultural Experimental Station and one half of it was poured into a concrete tank measuring 1 x 2 x 0.5 m (500 liters). 1000 ml of concentrated suspension (concentration degree 10^8 /ml) of the Shinjo strain, slope cultured for six days on the semi-synthetic agar culture medium, was added and stirred. This was left outdoors, but a lid was used to guard against rainwater and snow. A small volume of this was taken out each time as shown in Table 63, and the quantitative test was made by the phage method. The phage method was in accordance with the method 3, Figure 9.

2) Experimental Results and Observations

The viability of bacteria in the irrigation water during winter was 15 days, from 13 December to 28 December, as shown in Table 63.

Table 63

Viability of Bacteria in Irrigation Water during Winter

Area of Test	1958					1959	
	13 Dec.	14 Dec.	15 Dec.	18 Dec.	23 Dec.	28 Dec.	7 Jan. 15 Jan.
Irrigation Water in Winter	10,890 (2)	9,540 (1)	550 (0)	1,260 (0)	243 (0)	33 (0)	0 (0)
+ Bacterial Solution	1,808 (6)	16,925 (0)	910 (0)	8,690 (1)	957 (0)	92 (0)	0 (0)
Water Temperature °C	2.7	3.2	4.0	4.3	4.4	2.9	0.5 1.8

Note: Figures in the table stand for the volume of bacteria per 1 ml; figures in () stand for the volume of phage in the reservoir per 1 ml. Water temperatures were taken at 10 a.m., at the time of sampling.

Section 3. Overwintering of Bacteria in Cut Stems

1. The Life Cycle of Bacteria in Affected Cut Rice Stems

1) Test Method

The life cycle of bacteria was examined by the phage method on the cut rice stems from the affected rice paddies in the Shinyu area, Takada Municipality, Niigata Prefecture and in the Hokuriku Agricultural Experimental Station. The varieties of cut stems were Norin No. 29 (Shinyu Area) and Kinnanpu (Hokuriku Agricultural Experimental Station). For the latter, a comparison was made with the bacteria transferred on 2 November to a 1/5,000 pot and preserved in a greenhouse. The quantitative test of bacteria was conducted by the second method in Figure 9. Cut stems were dug out each time and the soil attached to them was eliminated by city water in order to prevent contamination, and they were crushed roughly with a large iron mortar. Two stems per sample were placed in a 1 liter wide-mouth jar, 300 ml of sterilized water added to this, and then it was shaken 500 times. They were placed in a thermostat at 28°C for about three hours and 50 ml of clouded bacterial suspension was taken and then centrifugally isolated at 2,000 rpm for five minutes. Foreign matter was eliminated, and the supernatant solution was used as the primary test solution.

2) Test Results and Observations

The life cycle of bacteria in cut stems from the foregoing samples.

As shown in Tables 64 and 65, the overwintering of bacteria on cut stems in Takada Municipality, Niigata Prefecture seemed to be difficult, and most bacteria died within the year. In this experiment the survival and detection of bacteria were recognized on the greenhouse preserved stems until the middle of February, but not thereafter.

Table 64

Bacterial Life Cycle in Infected Rice Stems
(Number of Bacteria per Stem)

Place	1958					1959			Remarks
	7 Nov.	8 Dec.	24 Dec.	15 Jan.	16 Feb.	1 Mar.	16 Apr.		
Shinyu Area,	0	70	125	0	0	0*	0*	0*	*Rotten and
Takada	1,670	0	0	0	0	0*	0*	0*	dead.
Municipality	1,540	0	0	0	0	0	0	0	Ground temper- ature at 10am.
Ground									
Temperature	12.7	6.0	4.8	0.7	0.5	2.9	10.2		
°C									

Note: The cut rice stems used for the quantitative test each time were collected each time; the continuous life cycle of bacteria in the same rice straws was not observed.

Table 65

Bacterial Life Cycle in Infected Cut Stems
(Number of Bacteria per Stem)

Place	1958				1959				Remarks
	2 Nov.	10 Dec.	23 Dec.	19 Jan.	16 Feb.	4 Mar.	17 Apr.		
Hokuriku District Agriculture Experimental Station	60 3,300	102 0	0	0	0	0	0*	* Rotten and dead	
Stems pre-served in greenhouse	634 18 1,976	906 1,128 0	91 305 130	101 62 0	8 467 0	0 0 0	0 0 0	Temperature in greenhouse: 16-26°C	
Ground Temperature °C	13.2	4.7	4.0	0.6	0.45	2.3	11.0		

Note: During the snow season of 1958-1959, no snow in December 1959, but snow from 16 January to 18 March 1959.

Table 66

Rate of Cut Rice Stems Overwintering Survival
Rate in the Hokuriku District

Place of Investigation	Rice Variety	Rate of Sprout Within the Year (%)	Rate of Overwintering of Survival Stem (%)	Date of Investigation
Kaminada, Takada Municipality, Niigata Pref.	Kinnanpu	88	0	28 April
" "	Hokuriku No. 62	91	0	"
Nagaoka Municipality, Niigata Pref.	Koshiji early	96	0	12 May
Akiba, Niitsu Municipality, Niigata Pref.	Yachikogane	-	0	2 May
Suzawa, Itoukawa Municipality, Niigata Pref.	Hokuriku No. 52	-	0	30 April
Tachiyama-cho, Arakawa-gun, Toyama Pref.	Kinnanpu	53	0	27 April
Anami-cho, Arakawa-gun, Toyama Pref.	Kiyosumi	-	0	24 April
Iida, Tamasu Municipality, Ishikawa Pref.	Kagaminori	92	0	31 March
Kawakita-gun, Ishikawa Pref.	Norin No. 1	95	0	2 April
Ishikawa Agricultural Experimental Station, Kanazawa Municipality, Ishikawa Pref.	Towada	-	0	16 April

Maruoka-cho, Sakai-gun, Fukuminori	-	0	4 May
Fukui Pref.	-	0	19 May
Bessho, Sanbo-gun, Sanin No. 17	-	0	11 March
Fukui Pref. " "	-	6	
" " Sanbonasahi	-	6	

Note: Figures stand for the number of stems which survived rot, and death per 100-200 stems.

2. The Rate of Overwintering Survival of Cut Rice Stems in the Hokuriku District

From March to May 1959, the overwintering survival of cut rice stems of the preceding year under natural conditions was investigated in several prefectures in the Hokuriku District. The results of investigation are shown in Table 66.

As shown in Table 66, because wet rice paddies are widely distributed and snow is heavy in winter, it was found that rice plant stems would not survive until the following spring.

Section 4. Overwintering of Bacteria in Affected Rice Stems

1. Life Cycle of Bacteria in Affected Rice Stems

1) Test Method

By storing infected rice stems (variety Kinnanpu) from Shimonoda, Takada Municipality, Niigata Prefecture in October 1958 on rice racks, outdoors and indoors, the life cycle of bacteria during the winter was examined according to the phage method. The phage quantitative method was done according to the third method, Figure 9. 20 leaf blades from stems were separated and cut into 1 cm strips. 50 ml of water was added to them and stirred, then centrifugally isolated at 2,000 rpm for three minutes. Foreign matter and pieces of tissue were eliminated, and the supernatant solution was used as the primary solution. Examination of the infected stems stored on the rice rack took place on 24 October, and on 25 October of those stored outdoors. Differentiation was made between the stems inside, in the case of outdoor storing, and the topmost stems.

2) Test Results and Observations

The life cycle of infected rice stems in winter was examined according to the phage method as shown in Table 67.

Table 67

Life Cycle of Bacteria in Stored Affected Rice Plant Leaves
(Number of Bacteria per 10 Infected Leaves)

Area of Test	1958				1959			
	26 Oct.	14 Nov.	25 Nov.	10 Dec.	26 Jan.	19 Feb.	6 Mar.	22 Apr.
Stored Indoor	252,160	876,930	16,330	111,240	25,810	36,450	51,430	92,820
On Rice Racks		1,210	760	0	0	0	0	0
Stored Inside		193,430	122,600	62,990	12,270	66,270	14,580	16,660
Stored Topside		76,160	119,660	0	8,330	0	0	0

Note: Figures are the means of three repetitions.

As shown in Table 67, the overwintering of bacteria in affected stems was completely possible until the following spring in the case of indoor storage; in the case of those stored outside, they showed almost no difference from those stored indoors, surviving until the following spring. In the case of storage on rice stacks and the topside stems stored outside, bacteria seemed to die within the year (the end of November).

2. Life Cycle of Bacteria in the Infected Stems Left in Fields

1) Test Method

Several bundles of infected rice stems (Sanin No. 52) from the infected rice paddies in Shimonoda, Takada Municipality, Niigata Prefecture were selected on 23 October 1958 and left on a path in rice paddies. The life cycle of bacteria in infected rice stems under the conditions of rain, wind, and snow was examined according to the phage method. The quantitative test was conducted according to the same method as in the preceding section, in accordance with the third method, Figure 9. As a comparison, some affected rice stems were kept in the laboratory for the quantitative test. Because of the inclement weather, due to snow after 22 January 1959, and because bacteria were not detected, the quantitative test was temporarily suspended.

2) Test Results and Observations

The results of the foregoing test are shown in Table 68.

As shown in Table 68, bacteria survived about 15 days in the stems left in the fields. Moreover, affected rice stems on 10 December were in a fragile condition; especially the leaf blades, were in such a condition that they were hardly able to serve as test samples.

Table 68

Life Cycle of Bacteria in Infected Stems
Abandoned in Field

① 試驗區	1958年②						1959年				
	③-④ 10月26日	10月31日	11月11日	11月25日	12月10日	12月26日	1月** 22日	2月 19日	3月 6日	4月 27日	5月 16日
⑤ 水田放棄	10,480*	450	33	0	0	0	中止	中止	中止	0	0
⑥ 被害菌	82,260*	4,820	705	0	0	0	中止	中止	中止	0	0
⑦ 屋內保存 被害菌		14,190 3,468	5,001 3,072	7,072 692	2,483 1,743	2,223 19,110	1,346 1,228	578 973	17 490	668 150	512 106
⑧ 野外10時氣溫	12.9°C	12.1	10.4	8.0	6.6	4.4	1.7	2.3	5.5	11.2	16.0

Note: *Volume of bacteria at the beginning of the test
**Almost rotten condition

[Legend]: 1) Area of test; 2) Year; 3) Month; 4) Day; 5) Infected stems abandoned in fields; 6) Suspended; 7) Infected stems stored indoors; 8) Outdoor temperature at 10 a.m.

Section 5. Overwintering of Bacteria in Rice

1. Life Cycle of Bacteria in Rice Seed

1) Test Method

A test was made on the rice seeds collected from the frequently infected rice paddies (Nihonkai variety) in the Sakon area, Nagaoka Municipality in 1958. Another test was made on the rice seeds collected from the frequently infected rice paddies (Minnanpu variety), in Mawamura Nishiki, Takada Municipality in 1959. On 12 October 1959, tests were made of the rice seeds threshed immediately after harvesting, and another group that were threshed after the rice stems had been naturally dried for 23 days on the rice racks.

Rice seeds were separated into chaff and unhulled rice by the threshing machine, and the life cycle of bacteria in them was tested according to the phage method in the periods shown in Table 67 to 69. The quantitative test followed the third method, Figure 9. In 1958, 50 gr of rice was scaled, and placed in a 500 ml flask with 100 ml

of sterilized water added and shaken 300 times. Then it was allowed to set in a thermostat at 28°C for four hours. Its rinsed bacterial suspension was the primary test sample solution. In the 1959 test, 20 grams of rice chaff were placed in a 500 ml flask, 160 ml of sterilized water added to this, and shaken 300 times. The surface rinsed solution of chaff was then allowed to set for three hours at room temperature. This was mixed with the remaining chaff which was roughly crushed with an iron mortar, and stirred after adding 100 ml of sterilized water, which was used as a test sample. Furthermore, foreign matter was eliminated from these test samples by centrifugal isolation at 2,000 rpm, for two minutes. The supernatant was used as the primary test sample solution. 50 grams of unhulled rice was shaken 300 times in sterilized water, and its rinse water was used as the primary test sample solution.

2) Test Results and Observations

The results of the investigation of the life cycle of bacteria in infected rice seeds by the foregoing method are shown in Tables 69-71.

Table 69

Life Cycle of Bacteria in Infected Rice Seeds
(1958 Rice Seeds) (Number of Bacteria
per 10 gr of Rice Seeds)

① 採種場所	1958年②				1959年			
	③ ④ 10月31日*	11月19日	11月25日	12月22日	1月29日	2月14日	3月7日	4月13日
⑤ 長岡市左近地区	412	0	0	0	0	0	0	0
	3,330	119	0	33	0	0	—	—

Note: *About 20 days after seed collection, and about one month after harvest.

[Legend]: 1) Place of Seed Collection; 2) Year; 3) Month; 4) Day; 5) Sakon area, Nagaoka Municipality.

Table 70

Life Cycle of Bacteria in Infected Rice Seeds (1959 Rice
Seeds, from Hokuriku District Agricultural
Experimental Station; Rice dried on
Rice Rack) (Number of Bacteria
per 10 gr of Rice Seed)

Area of Test	1959			1960			
	10 Nov.	22 Nov.	18 Dec.	16 Jan.	3 Feb.	12 Mar.	1 Apr. 26 Apr.
Chaff Rinsed Solution	20	0	0	0	0	0	-
Chaff Ground Solution	3,330	961	1,219	666	292	1,461?	0
Unhulled Rice Rinsed Solution	0	0	0	0	0	-	-

Note: *one month after harvest.

Table 71

Life Cycle of Bacteria in Infected Rice Seeds
(1959 Rice Seeds, from Hokuriku District
Agricultural Experimental Station, Rice
was Threshed Immediately After Harvest)
(Number of Bacteria per
10 gr of Rice Seed)

Area of Test	1959		1960			
	19 Nov.*	22 Nov.	18 Dec.	16 Jan.	3 Feb.	12 Mar. 1 Apr. 26 Apr.
Chaff Rinsed Solution	63	453	32	0	0	0 0 0
Chaff Ground Solution	12,710	2,530	1,144	986	603	1,186 0 0
Unhulled Rice Rinsed Solution			0	0	0	0 -

Note: *One month after harvest

As shown in Tables 69-71, the overwintering of bacteria in infected rice seeds seemed to last until the middle of March. It was further found that bacteria were latent in the tissues of inner and outer glumes, as shown in Tables 70 and 71.

Section 6. Overwintering of Bacteria in Host Plants

Description was made of the host range of the pathogen of rice bacterial leaf blight in Chapter V. At this point, investigation was made of the ecology of the rhizome of Leersia oryzoides (Linn.) Sw., regarded as the most important source of bacterial overwintering and infection, the germination of its seeds, and the morphological difference from Leersia japonica Makino. At the same time, the overwintering of bacteria in the rhizome in the natural vegetation area of Leersia oryzoides (Linn.) Sw. was examined according to the phage method.

1. Ecology of the Rhizome of Leersia oryzoides (Linn.) Sw. during Winter

In Niigata Prefecture, the above ground portion of the stem gradually fades, beginning in the early part of November, and seeds that grow on spikelets mature and fall off. Thereafter, the above ground portion falls with the drop in temperature and dies from the latter part of November to the early part of December. But since this is a perennial grass, its rhizome maintains a green color, and its root is filled and does not die out. By the latter part of February, under a snow cover new buds begin in the fore part of the rhizome and with the thaw in the latter part of March, the growth of the bud begins. In the middle part of April a new above ground stem and leaves develop. In April when the new leaves develop in the above ground portion, the rhizome gradually loses its green color and with its decline it turns yellow. It dies with the growth and development of the new rhizome.

2. Morphological Differences Among Leersia oryzoides (Linn.) Sw., Leersia oryzoides (Linn.) Sw. var. japonica (Ret) Honda and Leersia japonica Makino of Ear Formation

The classification of graminaceae is done by identification of ears. However, in the case of Leersia oryzoides (Linn.) Sw. which is the overwintering base of the pathogen of rice bacterial leaf blight and the source of the

Table 72

Morphological Differences Between Leersia oryzoides
(Linn.) Sw. and Leersia japonica Makino

Classification	<u>Leersia oryzoides</u> (Linn.) Sw.	<u>Leersia japonica</u> Makino
Natural Vegetation Ground	In many cases this naturally grows on river banks, the outer edges of large deltas, reservoirs, ditches, ridges and other shores with little water stagnation; it is found less in water-pool channels, ditches, and the center of and ponds.	In many cases it naturally grows in water at such places where water is pooled constantly as reservoirs, ditches, irrigation channels, ridges, and marshes.
Color of Leaf	Green-yellow	Blue-green
Color of Sheath	Same as above	Blue green, but partially colored deep red purple
Bristles in the Center Ribs of the Reverse Side of Leaf Blades	Protruding Bristles are formed	Without almost no bristles, and smooth.
Touch of the Bristles of Sheath	Hard bristles grow sparsely, with strong resistance to hand-touching	Soft hairs grow sparsely, and feel smooth to the touch.
Cilia on the edges of Sheath	No Cilia on one side of sheath edges	Clear-cut comb-tooth like cilia grow parallel on one side along the joints of sheath

Leaf Blade Edges	Saw-tooth like seta grow on leaf edge.	Leaf edges show only saw-tooth like notches.
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Table 73

Differences Between Leersia oryzoides (Linn.) Sw. and Leersia Oryzoides (Linn.) Sw. var. japonica (Ret) Honda

Classification	<u>Leersia oryzoides</u> (Linn.) Sw.	<u>Leersia oryzoides</u> (Linn.) Sw. var. <u>japonica</u> (Ret) Honda
Touch	Soft and weak	Rough and hard
Ceta on Sheath	Short ceta grow on sheath surface	Ceta growing on sheath surface are long and rough, and strong resistance is felt when rubbed.
Cilia on Leaf Edges	Short hairs and cilia grow on leaf edges.	Cilia growing on leaf edges are extremely long.
Ceta on Coryopsis Surface	Long ceta grow only on the vein ridge lines on the surface of inner and outer coryopsis, and in other parts are short protuberations.	Short bristles grow densely on both inner and outer coryopsis, and long ceta grow on ridge lines and other parts.
Size of Coryopsis and fructification	The size of the coryopsis is 8-9 mm at longest diameter, 1.5-2.0 mm wide. Most coryopsis fructification, with thickness	The size of the coryopsis is 7 mm at longest diameter, 2.0-2.5 mm, flat-shape. No fructification in the Hokuriku District.

infection of this disease, it resembles, before coming to ears, Leersia japonica Makino. This makes distinction difficult. Also, because there are many grasses that belong to Leersia oryzoides (Linn.) Sw. var. japonica (Ret) Honda^{96, 159, 166}, it is important to clarify their differences in the growth period before coming to ear in order to investigate the relationship between the overwintering of the pathogen of rice bacterial leaf blight and the primary infection. Therefore, the author investigated the morphology of Leersia oryzoides (Linn.) Sw. japonica (Rets) Honda, and Leersia japonica Makino, and compared it with Leersia oryzoides (Linn.) Sw. The main differences clarified through investigation, are as shown in Tables 72 and 73. (See photographs 29 and 30).

3. The Fructification and Germination of the Caryopsis of Leersia oryzoides (Linn.) Sw.¹⁶⁹

Leersia oryzoides is a perennial grass, but since it grows caryopsis resembling rice plants; it has the possibility of seed multiplication. However, according to observations of the author, the places and positions of the natural growth of Leersia oryzoides (Linn.) Sw. are fixed year after year, and no new groups develop. This seems to be due to the rarity of seed multiplication. Several morphological differences between Leersia oryzoides (Linn) Sw. var. japonica, (Rets) Honda and Leersia oryzoides (Linn) Sw. have already been described. Tests were made on the growth of the caryopsis of both grasses, their fructification, and on the caryopsis and germination of Leersia oryzoides (Linn) Sw. japonica, (Rets) Honda.

1) Investigation of Fructification of Leersia oryzoides (Linn.) Sw. and Leersia oryzoides (Linn.) Sw. var. japonica, (Rets) Honda.

In 1959-1960 observation was made of several colonies of Leersia oryzoides (Linn) Sw. and of Leersia oryzoides (Linn) japonica (Rets) Honda, growing in Takada Municipality, Niigata Prefecture. It was found that they all came into ears in September and October, and grew panicle spikelets. All the colonies of Leersia oryzoides (Linn) Sw. showed no fructification of caryopsis, while the percentage of fructification in the case of Leersia oryzoides (Linn) Sw. var. japonica (Rets) Honda was 93.5%. Based on the results of this investigation, further investigation was made of the colonies of Leersia oryzoides (Linn) Sw. growing in Takada, Niigata Prefecture, Naoetsu Municipality, Nakakeijogun, Kashiwazaki Municipality, Nagaoka Municipality, Nakakamabara-gun, Niitsu Municipality, Niigata Municipality,

Nyuzen-cho, Arakawa-gun, Toyama Prefecture, and Toyama Municipality. The results showed no fructification. (See photograph 27).

2) Germination Test of Leersia oryzoides (Linn) Sw.

In the latter part of November 1959, and on 23 November 1960, germination tests were made, using the seeds of Leersia oryzoides (Linn) Sw. var. japonica (Rets) Honda and Leersia oryzoides (Linn.) Sw. collected at the Hokuriku District Agricultural Experimental Station, in 15 cm unglazed pots and in bowls with filter paper. The results are shown in Tables 74 and 75.

Table 74

Results of Germination Tests of Leersia oryzoides (Linn.) Sw. in Unglazed Planters

<u>Leersia oryzoides</u> (Linn.) Sw.			<u>Leersia Oryzoides</u> (Linn.) Sw. var. <u>japonica</u> (Rets) Honda		
Planter Number	Number of Seeds sowed	Number Germinated	Planter Number	Number of Seeds sowed	Number Germinated
1	40-50	0	1	40-50	0
2	"	0	2	"	0
3	"	0	3	"	0
4	"	0	4	"	0
5	"	0	5	"	0

Note: Seeds produced in 1959, and sown at the Hokuriku District Agricultural Experimental Station (16 June 1960).

As shown in Tables 74 and 75, no germination was observed in the case of seeds with capsule caryopsis and inner caryopsis even after one month. Therefore, the caryopsis skin was excoriated and the germination test was conducted on water-absorbed filter paper in a bowl. On this occasion, some rinds close to embryos were cut with knives. The results are as shown in Table 76.

Table 75

Fructification and Germination of Leersia oryzoides
(Linn.) Sw. (Investigated on Ears of 1959)

<u>Leersia oryzoides</u> (Linn.) Sw.				<u>Leersia oryzoides</u> (Linn.) Sw. var. <u>japonica</u> (Rets) Honda			
Ear	Grains per Ear	Number of Fructification of the Same	Number Germinated in Bowl	*			
				Ear	Grains per Ear	Number of Fructification of the Same	Number Germinated in Bowl
1	58	56	0	1	82	0	0
2	62	55	0	2	96	0	0
3	132	127	0	3	77	0	0
4	66	61	0	4	132	0	0
5	122	107	0	5	129	0	0
6	43	40	0	6	65	0	0
7	32	30	0	7	113	0	0
8	45	41	0	8	187	0	0
9	43	37	0	9	67	0	0
10	119	111	0	10	54	0	0
11	87	85	0	11	68	0	0
12	86	82	0	12	96	0	0
13	97	93	0	13	43	0	0
14	79	74	0	14	132	0	0
15	72	62	0	15	95	0	0
16	66	57	0	16	35	0	0
Total	1,209	1,130 (93.5%)		Total	1,421	0 (0%)	

Note: *Number germinated per 20 grains. Leersia oryzoides (Linn.) Sw. seeds only
swelled and did not germinate. (24 June)

Table 76

Germination Test of Leersia oryzoides (Linn.) Sw.
(In a thermostat at 28°C)

Bowl No.	I	II	III	IV	V	VI	VII
Seed Treatment							
Uncut Seeds	0	0	0	0	0	0	0
Cut Seeds	18	12	17	11	16	20	19

Note: Sample seeds were collected in 1960 at the Hokuriku District Agricultural Experimental Station, and experiments were made from 26 November to 5 December.

As shown in Table 76, uncut seeds absorb water and their embryos swell. But no germination was observed in three weeks probably because of the thickness of rinds. Against this, rind cutting resulted in 81% germination (See photograph 28).

4. Overwintering Cycle of Bacteria in Rhizome of Leersia oryzoides (Linn.) Sw. var. japonica (Rets) Honda

1) Test Method

Several colonies of Leersia oryzoides (Linn.) Sw. var. japonica (Rets) Honda (whose summer infection was confirmed) growing in rice paddy ridges, ditches and water canals around the Hokuriku District Agricultural Experimental Station, from November 1959 to May 1960, were designated as investigation points. Their rhizomes were dug up on the dates shown in Table 77, and the detection and quantitative test for bacteria was made. The quantitative test for bacteria followed the third method, Figure 9, and each time 200 gr of the rhizome of Leersia oryzoides (Linn) Sw. var. japonica (Rets) Honda were used as the test sample. The rhizome was rinsed after it was dug up to eliminate soil attached to it, crushed in an iron mortar, and then placed in a 100 ml specimen glass. 500 ml of sterile water was added to this and it was closed tightly and shaken 500 times, then allowed to set in a thermostat at 30°C for two hours. After that its supernatant was centrifuged and isolated at 2,000 rpm. for two minutes. After eliminating grains of soil and foreign matter, it was used as the primary test sample solution.

2) Test Results and Observations

The life cycle of bacteria during winter of the rhizome of Leersia oryzoides (Linn.) Sw. var. japonica, (Rets) Honda was examined by the foregoing method, with the results as shown in Table 77. Table 78 shows the results of the measurement of the ground temperature of the rhizome at each investigation point.

Table 77

Life Cycle of Bacteria in the Rhizome of
Leersia oryzoides (Linn.) Sw. var.
japonica (Rets) Honda

① エソノサヤヌ カダサ採集地 点	1959年①			1960年								② 地上部葉 葉におけ る初発月 日
	11月 19日	12月 10日	12月 17日	1月 6日	1月 19日	2月 10日	3月 19日	4月 10日	4月 28日	5月 16日	5月 29日	
③ 新潟県高田市 安子	871	221	1,975	15	33	0	0	0	0	688	78 (193)	7月18日
④ 茨 沢	9,670	104	1,713	620	0	0	11	0	0	1,102	4,862 (24,160)	6月10日
⑤ 戸ノ目	1,020	75	996	0	0	0	0	0	0	1,470	1,312 (24)	7月2日
⑥ 上 稲 田	1,236	195	0	0	51	0	9	0	0	280	149 (155)	7月13日
⑦ 北陸農試内	80	0	0	0	0	0	0	0	0	1,190	5,244 (810)	6月28日

Note: Figures in the table show the number of bacteria per 100 gr of the rhizome of Leersia oryzoides (Linn.) Sw. var. japonica, (Rets) Honda. Figures in () on 29 May show the number of bacteria per 20 gr of above ground stem leaf.

[Legend]: 1) Collection point of Leersia oryzoides (Linn.) Sw. var. japonica (Rets) Honda; 2) Date of first occurrence in the above ground stem leaf; 3) Koyasu, Takada Municipality, Niigata Pref.; 4) Ibarazawa, Takada Municipality, Niigata Pref.; 5) Tonome, Takada Municipality, Niigata Pref.; 6) Kamiinada, Takada Municipality, Niigata Pref.; 7) Hokuriku District Agricultural Experimental Station; 8) Year; 9) Month; 10) Day.

Table 78

Ground Temperature of the Rhizome of *Leersia oryzoides*
(Linn.) Sw. (November 1959-May 1960) at
Investigation Points

Date of Measurement of Ground Temperature	Koyasu, Takada Municipality Niigata Pref.		Ibarazawa, Takada Municipality Niigata Pref.		Tonome, Takada Municipality Niigata Pref.		Hokuriku Agricultural Experi- mental Station	
	°C		°C		°C		°C	
11.19	8	6.9	6.6	7.7	7.9	-	3 cm on	
12.10	4.5	5.5	5.2	4.6	6.6	7 Dec.	20	
27	0.5	0.4	1.0	0.5	4.5		7.2	
1. 6	1.0	1.9	1.4	1.7	0.5		6.0	
19	0.3	0.5	0.8	0.8	1.3		50.0	
2.16	0.5	0.5	0.2	0.5	0.8		11.5	
3.19	2.9	3.3	3.1	2.3	4.5		21 Mar	
4.10	11.0	10.5	11.0	10.2	13.1			
28	13.2	12.5	12.8	13.9	12.6	-		
5.16	19.0	19.5	17.9	19.7	16.0	-		
29	18.8	19.0	18.1	18.5	16.7	-		

Note: Observation at 4-5 cm underground, at 10 a.m. The snow season: 27 December 1959 to 3 April 1960.

As shown in Table 77, bacteria were detected in the rhizome of the Leersia oryzoides (Linn.) Sw. in Niigata Prefecture from November to the early part of the following January. But for three months from February when new stem leaves develop to the latter part of April, bacteria were not detected for a while, which led to the conclusion that bacteria died during this period. However a large volume of bacteria were detected suddenly in the middle part of May. Therefore, it seems that bacteria colonies in fall decrease to such an extent that detection of bacteria is difficult, but they continue to survive in the rhizome and increase in number with the rise in ground temperature in the latter part of April (ground temperature at 10 a.m. in May surpasses 15°C). From the foregoing results, there is a good possibility that the primary infection is carried out from the numerous colonies of Leersia oryzoides (Linn.) Sw. var. japonica (Rets) Honda around the ridges, water canals, and rivers in the Hokuriku district through the medium of irrigation water during the rice seedling period from the latter part of April to the latter part of May.

5 The Overwintering Cycle of Bacteria in the Rhizome of Host Grasses Other than Leersia oryzoides (Linn.) Sw.

1) Test Method

The life cycle of bacteria overwintering in the rhizomes of Ziznia latifolia Turcz, and Leersia oryzoides (Linn.) Sw. that grow naturally in the fields of the Hokuriku District Agricultural Experimental Station was investigated from November 1959 to May 1960. The quantitative method followed the previous one. For Ziznia latifolia Turcz, 500 gr of the test sample were taken, and 300 ml of sterile water was added.

2) Test Results and Observations

The results of the investigation of the life cycle of bacteria during winter on the rhizome of host plants conducted in the aforementioned method are shown in Table 79.

Table 79

Life Cycle of Bacteria in Rhizomes of Host Grasses

Date of Investigation		1959					1960				
		19 Nov.	9-10 Dec.	6-9 Jan.	16-18 Feb.	19-20 March	28 April	1 May	16-18 May	29-30 May	
Host Plant											
<u>Zizania latifolia</u> Turcz		12,340	513	0	0	0	0	0	8	0	
		415	4,620	0	0	0	0	0	101	0	
<u>Leersia japonica</u> Makino		978	108	0	0	0	0	0	0	0	
		195	92	0	0	0	0	0	0	0	
<u>Isachne globosa</u> (thunb) O. Kuntze		0	0	0	0	0	0	0	22	0	
		13	0	0	0	0	0	0	0	1	
<u>Leersia oryzoides</u> (Linn.) Sw. var. <u>japonica</u> (Rets) Honda		10,350	8,310	0	0	0	0	0	0	1,970	
		10,050	14	0	0	0	0	0	0	691	

Note: Figures in the table show the volume of bacteria per 100 gr of rhizome.

As shown in Table 79, the contamination and maintenance of bacteria were detected in Ziznia latifolia Trucz, Leersia japonica Makino as in Leersia oryzoides (Linn) Sw. var. japonica (Tets) Honda from November to the early part of December, but no bacteria were detected from January to April. In the case of Isachne globosa (Thunb) O. Kuntze, a small amount of bacterial contamination was noticed in November, and no bacteria were detected until the contamination caused by the flow of irrigation water in the following spring. However, in the case of Ziznia latifolia Turcz, a considerable volume of bacteria was detected for a while in the middle part of May, the following spring. In the quantitative test in the latter part of May none were detected. Therefore, it was not clear whether or not bacteria continued overwintering in the rhizome as in the preceding year, as in the case of Leersia oryzoides (Linn.) Sw. It was further concluded from the results of this test that in Leersia japonica Makino and Isachne globosa (Thunb) O. Kuntze there was no overwintering of bacteria in the rhizome.

6. The Early Infection Period of Leersia oryzoides (Linn.) Sw. in Niigata Prefecture

Because there had been no record kept on the infection of Leersia oryzoides (Linn.) Sw., investigation was conducted from 1958 to 1960 on the Leersia oryzoides (Linn.) Sw. colonies in regard to the periods of infection, with results as shown in Table 80.

Table 80

The Early Infection Period of Leersia oryzoides
(Linn.) Sw. in Niigata Prefecture

Year	Place		Date of discovery of initial infection	Growth phase of rice at the time of discovery	Initial infection period of rice
1958	Kamiinada, Takada Municipality, Niigata Pref.		27 June		
	Shimonoda, Takada Niigata Pref. (I)	"	29 June	One month after transplanting	
	"	" (II)	"	"	
	Koyasu, Takada Municipality, Niigata Pref.		2 July		Mid-July
	Gennyu, Maoetsu Municipality, Niigata Pref.		1 July		
	Sanai, Maoetsu Municipality, Niigata Pref.	"	"	Intermediate and peak tillering period	
1959	Koizumi, Niigata Pref.	"	"	"	
	Aono, Niigata Pref.	"	"	"	
	Kamiinada, Takada Municipality, Niigata Pref.		21 June	Peak tillering period (early maturing)	
	Koyasu, Niigata Pref.	"	"	Intermediate till- ering period	
	Aono, Maoetsu Municipality, Niigata Pref.		10 June	Early tillering period	24 June to early July

	Nagakura-cho, Nagaoka Municipality, Niigata Pref. Koshiji-cho, Nishima-gun, Niigata Pref.	23 May 29 May	Early tillering period 24 June to early July
1959	Ikegahara, Kochitani Municipality, Niigata Pref. Korimaki-cho, Nishikamabara-gun, Niigata Pref. Ozeki, Niitsu Municipality, Niigata Pref.	9 June 30 June 17 June	Intermediate tillering period
	Kaminada, Takada Municipality, Niigata Pref. Ibarazawa, Niigata Pref. Koyasu, Niigata Pref.	29 June 10 June 15 July	Intermediate tillering period Early tillering period Peak tillering period
1960	Nagakura-cho, Nagaoka Municipality, Niigata Pref. Kamimaejima, Nagaoka Municipality, Niigata Pref. Ozeki, Niitsu Municipality, Niigata Pref.	4 June " " 1 June	Mid-July Ten days after transplanting
	Korimaki-cho, Nishikamabara-gun, Niigata Pref.	6 July	Intermediate tillering period

As shown in Table 80, the period of natural infection of Leersia oryzoides (Linn.) Sw. in Niigata Prefecture varies with the year. It seems that the infection is discovered in the latter part of May at the earliest, and by the early part of July at latest. The investigation for a three year period revealed that the average time of infection was in the latter part of June.

Section 7. Results of Past Tests on the Overwintering of the Pathogen of Rice Bacterial Leaf Blight

1. Overwintering in Soil

Ishiyama⁴⁵ reported that the pathogen of this disease exists in soil before rice transplanting because bacteria are isolated from the surface soil of rice paddies before transplanting in constantly infected areas and that bacteria survive in the sterile soil in an unglazed cylinder from the latter part of November to the latter part of the following April. In this experiment, bacteria were isolated in seven out of 10 soil samples before transplanting; a high percentage of isolation. For this reason until several years ago it was thought that this pathogen would survive and overwinter in soil. Thereafter, Inoue, et al^{43,44} attempted similar isolation of bacteria from the soil of constantly infected areas, but without definite results. Wakimoto¹³⁹ attempted to detect bacteria in soil by the phage method, with negative results. Seki, et al¹⁰⁸ observed the existence of bacteria for less than a month in the soil of infected areas, and a six month period in rice straw manure. Recent views hold that the period of bacterial viability is extremely short, and the possibility of overwintering is also negatively viewed. In the experiment conducted by the author, bacteria had a short life under temperature conditions of more than 19°C, but at outdoor temperatures during winter, bacteria could survive for 50 days and in a refrigerator at 0-5°C for 26 days. However, these results must be verified further.

2. Overwintering in Intermediate Host Plants

Goto, et al²⁷⁻³⁰ conducted needle inoculation tests on the host range of the pathogen of rice bacterial leaf blight on 41 kinds of grasses naturally growing near rice paddies. They observed clear infection in Ziznia latifolia Turcz., Phalaris arudinacea Linn., and Leersia oryzoides (Linn) Sw. and lightly in Phragmites communis Trin, and

Isachne globosa (Thunb) O. Kuntze, thus re-isolating bacteria and identifying them. They further, as described already, recognized natural infection in Zizania latifolia Turcz, Leersia oryzoides (Linn.) Sw. and investigated the interrelationship with the infection of rice plants. Then, Inoue, et al⁴⁴ conducted the primary infection test of Leersia oryzoides (Linn.) Sw. by ploughing immediately prior to cultivation and transplanting into an irrigation channel, to verify this. This clarified the importance of the source of infection. However, the overwintering of bacteria in these grasses was not clear. So, Wakimoto,¹³⁹ Watanabe, and Kurita¹⁴⁷ ascertained by the phage method the survival and overwintering of bacteria until the following spring in rhizome which maintain a green color. Also Tagami, et al¹⁷⁰ conducted overwintering tests by bacterial inoculations on Leersia, japonica, Makino, Zizania latifolia Turcz, Phalaris arundinacea Linn. and Phragmites communis Trin with negative results.

3. Overwintering in Irrigated Water

Inoue, et al⁴⁴ attempted to isolate bacteria from the stagnant water in channels and rice paddies in constantly infected areas, but could not detect bacteria. Tagami, et al⁶⁹ detected the viability of this pathogen in irrigation water, and further observed that pathogens rapidly died and decreased within 36 hours, to less than 1%, but the decrease thereafter was extremely small. The author observed that the period of viability of the pathogens outdoors during winter was less than 25 days.

4. Overwintering in Infected Cut Stems

Tagami, et al^{70,123} tested and proved that infected cut rice stems serve as the base for overwintering of this pathogen. However, for the overwintering of bacteria, the survival of cut stems until the following spring is a prerequisite, and this is limited only to the dried fields that cultivate late maturing varieties in comparatively warm areas. On this point Nakazawa, et al⁸⁸ makes the same observation. However, in the tests and investigations of the author, the significance of cut stems as the base of overwintering was rather small compared with warmer areas, because the overwintering of cut stems themselves was impossible in the Hokuriku District.

5. Overwintering in Infected Leaves

Goto, et al³² considered the possibility of overwintering of bacteria in infected straw, because when infected

straw was ploughed into a nursery immediately before sowing seeds, seedlings from this nursery showed a considerable percentage of occurrence in the transplanted field. Thereafter, Inoue, et al⁴⁴ ascertained that the isolation of bacteria from infected straw is possible even after five months. Further, Tagami, et al⁷⁰ carried out quantitative tests for bacteria according to the phage method on infected straw stored indoors for eight months after harvest. In this respect, the author's investigation similarly revealed survival of more than six months in the straw stored outdoors and indoors.

6. Overwintering in Infected Rice Seeds

Goto, et al³² observed a strong trend of infection among affected unhulled rice after it was sowed, raised and transplanted to main fields, and suggested the possibility of the overwintering of bacteria in unhulled rice seeds. Thereafter, Wakimoto, et al³⁷ reported the survival of bacteria until the following mid-May or mid-June, thus proving the overwintering of bacteria in rice seeds. As has been previously stated the author also used a similar method, but only detected bacteria in the ground area of the chaff of rice seeds, thus with different survival period results. The differences seem to be differences in the degree of infection of unhulled rice and conditions of storage. Some tests have been made on the infection of rice seeds but without definite proof of infection.

7. Overwintering of Bacteria in Second Crop Plants

After the rice harvest, in two crop a year fields, various second crops are raised. These second crops have a high contamination potential by the pathogen as in the case of grasses in the field ridges. Inoue, et al⁴⁴ attempted direct isolation of overwintering bacteria from the roots of barley, wheat, and rape-seed, Wakimoto³⁹ attempted detection of bacteria according to the phage method in the roots of rape-seeds, wheat, broad beans, Suzumenoteppo, but all with negative results. However, Wakimoto* recognized the concentration of the pathogen in the roots of parsley, mizosoba, tsuyukusa, hokigiku and observed that it did not differ among different plants. Minakami⁸⁴ recognized a marked warming of the pathogen, as in rice, the roots of Phalaris arundinacea Linn, Mizosoba, Leersia Oryzoides (Linn.) Sw. From these facts it can be said that some role is played by second crop plants in overwintering and survival of the pathogen of rice bacterial leaf blight.

* Wakimoto Satoru (1957): Relationship between plant roots and the pathogen of rice bacterial leaf blight. Bulletin of Kyushu Disease and Insect Research Association, 3, 2-5.

Kiriu, et al⁵⁰ investigated the relationship between occurrence in second crop plants and primary crop fields, and recognized that there was a stronger trend of occurrence in the cases of lotus flowers, broad beans, and raddishes for seed collection purposes than in the case of wheat or rye. He conjectured that this was related to the volume of nitrogenous fertilizer in the second crop plants.

Section 8. The Overwintering of Dried Ooze and Its Value as a Source of Infection

Chapter VII, covered the past research on the overwintering of the pathogen of rice bacterial leaf blight and the test results of the author. These results showed the long period of survival of the pathogen in infected straw and unhulled rice under the dry conditions. In this respect, Wakimoto, et al¹⁴⁰ regarded that bacteria themselves as having the morphology to endure adverse conditions, including dryness. Thus tests were conducted on drying treatments of various kinds. They pointed out group dryness of bacteria as a specific condition necessary for a long survival. And at the same time, they reported, from results of tests on infected straw that even if bacteria are dried in groups, if they frequently contain water during winter, they gradually die out.

The author¹⁶⁶ pointed out that as a typical sample of the dried matter of this pathogen there was bacterial ooze secreted and formed on the leaf edges in the diseased section of the affected leaves. The author used this bacterial ooze in the test of viability and described the results. When this dried bacterial ooze is exposed to wind and rain, it is dried and hardened as lymphatic liquid, stuck to stems, and part of it falls to the surface of rice paddies. While the dried bacterial ooze stored in rice stems still maintain pathogenicity even after one year, that which falls on to the rice paddies gradually dies out. However, as has been described earlier, it does not easily absorb water in the wet soil, nor does it lose shape. It survives about six months. In the process of drying the heightening of electron density of bacterial cells themselves is observed. For this reason, the author estimated that bacterial structural changes are related to the long period of survival.

Generally, it has been pointed out that the covering and protective action of macro-molecule matter such as the capsule-like membrane, mucous layer, and rubber-like

bacterial ooze have a great impact on the survival of bacteria in bacterial diseases. Minakami⁸⁴ has also reported that when the pathogens are dried in leaf tissues, they are in a very stable condition due to the protective multi-glucose layer that the bacteria themselves have secreted. In addition to these there are numerous samples of tests that show that dryness has a decisive influence on the survival of dried bacterial ooze for long periods of time. Parker,⁹⁹ Hildebrand, et al³⁷ have pointed out the intimate relationship between the viability of Bac. amylovorus and dryness; Massey,⁷⁶ between dryness and Bact. malvacearum; and North⁹⁴ between dryness and Bact. vascularum. Recently, Hedrick³⁶ McKinney,⁷⁷ Leach,⁷¹ Crossan¹² examined the classification of multi-glucose, which is a component of dried bacterial ooze, and made research on the relationship between ooze and the perpetuating structures of bacteria, and suggested that dried bacterial ooze in bacterial disease is important for overwintering survival. In this respect, in the case of rice bacterial leaf blight, the dried bacterial ooze that is formed on infected leaves constitute convenient overwintering forms. (See photograph 31).

Section 9. General Summary

Description has been made of the test results obtained by the author on the overwintering of the pathogen of rice bacterial leaf blight and past research achievements. The test results obtained by the author on the survival period of this pathogen are summarized in Figure 10. The infection cycle of this disease, based on these results, is diagrammed in Figure 11.

In addition to the results shown in the Figures, the following test results have also been obtained.

1) A high electron density has been observed in the bacteria of dried bacterial ooze so that they can cope with adverse environment (especially dryness).

2) The overwintering survival rate of cut stems in the Hokuriku District is almost 0% with the exception of part of Fukui Prefecture. Most of them rot and die. Thus overwintering bacterial survival in cut stems is difficult.

3) Bacterial overwintering in rice seeds is carried out mostly in the tissues of the inner and outer paleas.

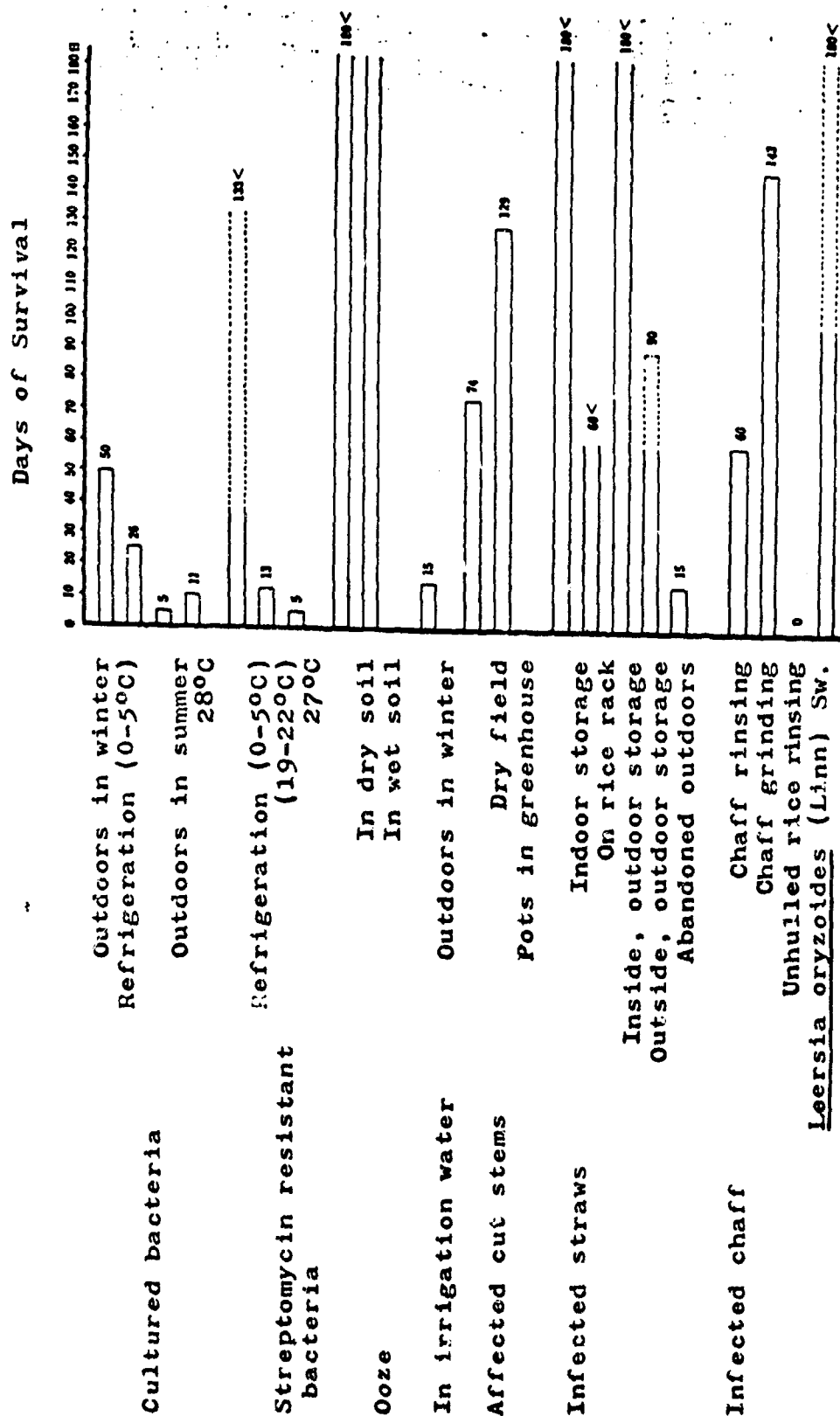


Fig. 19 Overwintering Locations and Length of Survival of the Pathogen of Rice Bacterial Leaf Blight (...stands for uncertain ones)

4) The rhizo-spheres of Leersia oryzoides (Linn.) Sw. var. japonica (Rets) Honda, an intermediate host plant, in winter, have full tissues and maintain a green color. And further, it was observed that during February under snow, new buds appeared on nodes, and with the thaw in March, the development of new buds began. By mid-April new over-ground stem leaves developed, and the rhizo-spheres during overwintering weakened and died.

5) A few morphological differences among Leersia oryzoides (Linn) Sw. var. japonica, (Rets) Honda, Leersia oryzoides (Linn.) Sw. Leersia japonica Makino before ear formation were investigated. For Leersia oryzoides (Linn.) Sw. the fructification of coryposis and germination were tested, and the difficulty of seed multiplication was clarified.

6) It was found out from the investigations conducted in 1958-1960, that the disease occurrence of Leersia oryzoides (Linn.) Sw. in Niigata Prefecture is generally from late-June to early July.

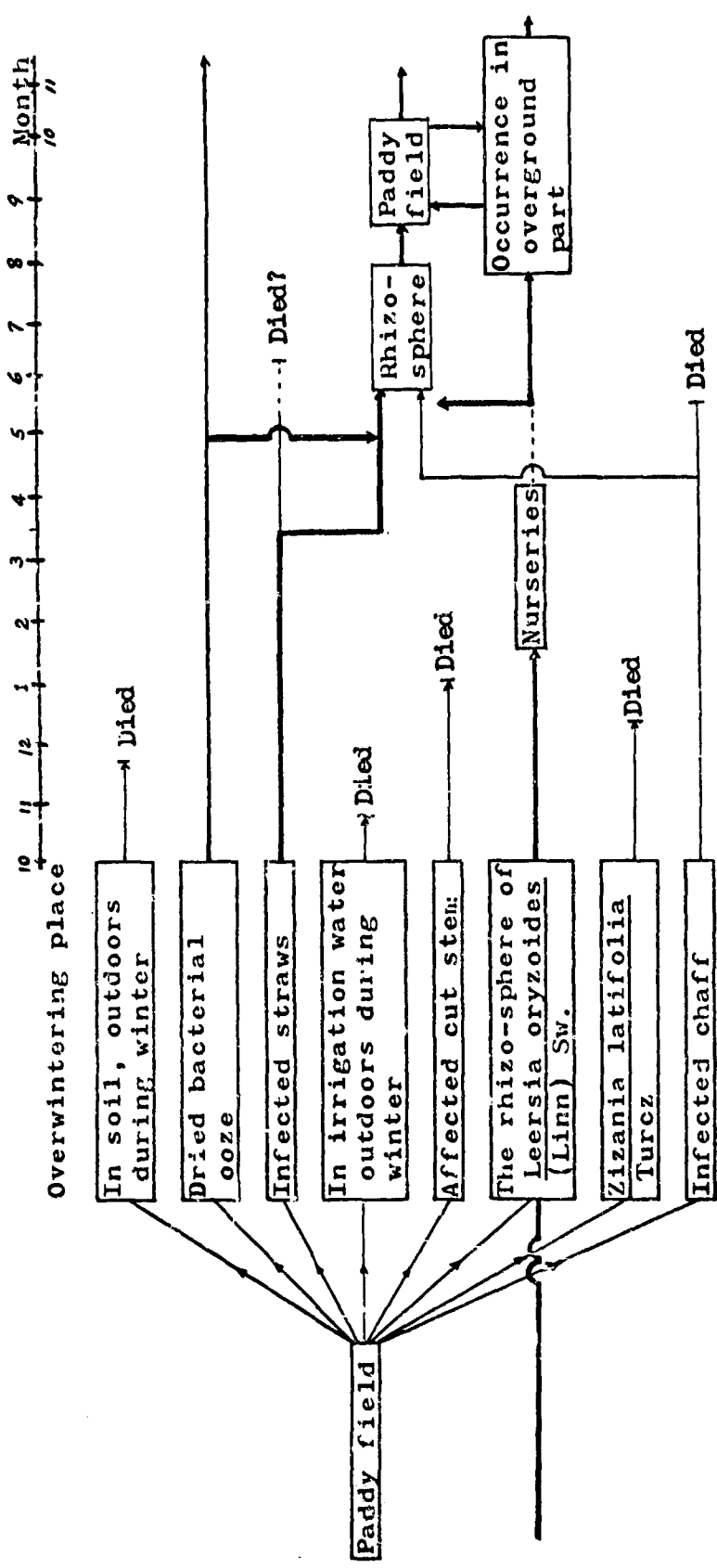


Fig. 11 Infection Cycle of the Pathogen of Rice Bacterial Leaf Blight in the Hokuriku District

CHAPTER IX. OCCURRENCE AND LIFE CYCLE OF RICE BACTERIAL LEAF BLIGHT DURING THE RICE GROWING PERIOD

Section 1. Occurrence and Life Cycle of Rice Bacterial Leaf Blight in the Hokuriku District

1. Disease Occurrence in Nurseries

Investigation was made for three years, from 1958 to 1960, in order to determine the disease outbreak in the nursery period in an attempt to clarify the occurrence morphology of rice bacterial leaf blight in nurseries.

1) Investigation Method

A patch was selected from the nurseries in Itouokawa Municipality, Nishi-keijo-gun, Takada Municipality and Naka-keijo-gun of Niigata Prefecture, and the disease outbreak in seedlings was investigated at these three patches (average 30 cm² per patch).

2) Investigation Results and Observations

Investigations were conducted in 25 places and at 223 points in 1958, 16 places and 91 points in 1959, and eight places and 35 points in 1960. But no occurrence in nurseries was observed in the Joetsu area of Niigata Prefecture.

2. Investigation of Disease Occurrence in Nurseries in the Prefectures of the Hokuriku District

For two years beginning in 1958, investigation was made in each prefecture of the Hokuriku District for rice bacterial leaf blight (joint and coordinated investigation by four related prefectures of the Hokuriku District). Especially in this investigation, disease outbreak in nurseries was examined. Especially in 1959, investigation was conducted by the use of questionnaires in 100 nurseries. The results of the investigation showed no occurrence had

been observed in any prefecture. It is believed that there was probably no occurrence in nurseries in the Hokuriku District.

3. Relationship Between the Source of Seedlings and Disease Occurrence

There are a few test samples in the warm south-west area on the relationship between the source of seedlings and the disease occurrence after transplanting rice to main paddies. In this section, the results of investigation made in 1958 and 1959 in the Hokuriku District, especially in Niigata Prefecture are given.

1) Investigation Locations and Methods

Seedlings were given to us by other cultivators due to the occurrence of yellowish-wilting or the shortage of seedlings at Aomi-cho, Nishi-keijo-gun, Itouokawa Municipality; Keijo-mura, Nika-keijo-gun; Miwanishiki-mura; Shita-borinouchi, Takada Municipality; Tomikawa; and the oil field in the latter. After these seedlings were transplanted in paddy fields, there was a considerable number of occurrences of rice bacterial leaf blight. The timing of the outbreak of the disease was about one month after transplanting, and in conjunction with disease occurrence at the sources, the occurrences were clearly determined to be by the infection of seedlings.

2) Results of Investigation and Observations

The results of investigation are shown in Table 81.

As shown in Table 81, the relationship between the source of seedlings and the disease outbreak in paddy fields is clear in the Joetsu area of Niigata Prefecture, and there seems to be a hazard of seedling infection.

4. Occurrence and Life-cycle of Rice Bacterial Leaf Blight in Niigata Prefecture

In order to clarify the occurrence and life cycle of rice bacterial leaf blight in Niigata Prefecture, the following occurrence process was investigated in the rice paddies near Takada Municipality.

1) Investigation Locations and Methods

Investigation was made on the number of infected stems during the growth period at paddies of the Hokuriku

Table 81
Instances of Seedling Infection in Niigata Prefecture

Investigation Location	Year of Investigation	Disease Occurrence from Seedling Infection in Main Paddies (Investigated first part of July)	Remarks
Front of Suzawa Primary School, Aomi-cho, Nishi-keijo-gun, Niigata Pref.	1958	-	Transplanting at all points in late May. Heavy rain fell during the nursery period in 1958, and most were immersed.
Tara-jima, Itoukawa, Niigata Pref.	"	-	
Hyakken-cho, Keijo-mura, Naka-keijo-gun, Niigata Pref.	1959	+	
Miwamuranishiki, Nakakeijo-gun, Niigata Pref.	"	+	
Tomeji, Aono, Naoetsu Municipality, Niigata Pref.	1958	+	
Shitagennyu, Naoetsu Municipality, Niigata Pref.	"	-	Considerably clear cases of seedling infection were observed in 1959 and 1961.
Horinouchi, Takada Municipality, Niigata Pref.	"	+	
Shimotomikawa, Takada Municipality, Niigata Pref.	1959	+	
Olfeld, Takada Municipality, Niigata Pref.	1961	+	

District Agricultural Experimental Station, Takada Municipality, Niigata Prefecture and several paddies in Takada Municipality as shown in Table 82, and the process of the disease was observed.

2) Results of Investigation and Observations

Results of investigation are shown in Table 82.

Table 82

Yearly Occurrence Process of Rice Bacterial Leaf Blight in Takada Municipality, Niigata Prefecture

① 年次	② 調査場所 (高田市)	③ 品名	④ 6 ⑤月			7 月			8 月			9 月			⑨ 備考
			⑥ 上	⑦ 中	⑧ 下	上	中	下	上	中	下	上	中	下	
1958	⑩ 北陸農試	⑪ 越後ネパール					+		+	+	+		+	+	⑫ 平年発生 稲作後半 とくに遅 展
	⑬ 下郷之内	⑭ 越 栄					+		+	+	+		+	+	
	⑮ 鴨 島	⑯ 北陸 52 号					+		+	+	+		+	+	
	⑰ 子 安	⑱ 山陰 52 号					+		+	+	+		+	+	
		⑲ 北陸農試	⑳ 気温 (最高) (°C)	㉑ 最低	㉒ 台風強度 (mm)	㉓ 積算 (mm)									
1959	北陸農試	㉔ シンマサリ	0	0			3	3	5	5		16	18	24	㉕ 早・多発 年 ㉖
	下野田	㉗ 金南風					+	+	+	+		+	+	+	
	下宮川	㉘ 越 栄					+	+	+	+		+	+	+	
		㉙ 山陰 52 号					+	+	+	+		+	+	+	
		㉚ 北陸農試	㉛ 気温 (最高) (°C)	㉜ 最低	㉝ 台風強度 (mm)	㉞ 積算 (mm)									
1960	立 町	㉟ 越 栄		0	0		0	0.1	0.5			0.9	1.5		㉕ 遅・少発 年 ㉖
	戸野目	㊱ "		0	0		0	0	0.1			0.1	0.1		
	茨 沢	㊲ "		0	0.1		0.1	1	2.2			3.3	6.9		
	鴨 島	㊳ "		0	0		0	0.2	0.2			1.6	3.6		
		㊴ 北陸農試	㊵ 気温 (最高) (°C)	㊶ 最低	㊷ 台風強度 (mm)	㊸ 積算 (mm)									

Note: + n shows the degree of occurrence by personal observation. Figures only show the percentage of infected stems (%).

[Legend]: 1) Year; 2) Place of investigation; 3) Variety; 4) Climatic elements; 5) Month; 6) First 10 days of month; 7) Middle 10 days of month; 8) Last 10 days of month; 9) Remarks; 10) Hokuriku Agricultural Experimental Station; 11) Echigo glutinous; 12) Occurrence as usual, and particular progress in the latter part of the rice growth period; 13) Shimoborinouchi; 14) Echiei; 15) Kamojima; 16) Hokuriku No 52; 17) Koyasu; 18) Sanin No 52; 19) Temperature; 20) Maximum; 21) Minimum; 22) Frequency of typhoons; 23) Accumulated precipitation; 24) Ginmasari; 25) Year of early and frequent occurrences; 26) Kinnanpu; 27) Shimonoda; 28) Shimotomikawa; 29) Tachi-machi; 30) Year of late and few occurrences; 31) Tonome; 32) Ibarazawa.

Table 82, shows the occurrence process for a three year period, first occurrence observed in mid-June at earliest, or in mid-July in usual years, and thereafter the degree of the disease progresses. The rate of the increase is higher in earlier occurrences with higher degrees of occurrence at the time. There seems to be close correlation between the time of the first occurrence and the degree of occurrence thereafter. On this point Kiriu, et al⁵² already made a report based on the results of detailed investigation and analysis of the damage caused by this disease. Similar trends seem to be observed in Hokuriku District, too.

It was also generally observed that the disease makes rapid development in mid and late August. Although the degree of progress of the disease in the latter half of rice growth is related to the degree of the first occurrence as mentioned above, it seems that it is also closely related to the climatic environment. That is, according to the investigation conducted by the author, judging from the climatic conditions of 1958 when the progress of the disease was remarkable in the latter half of rice growth, there seemed to be close correlation between the frequency of rain, totaling over 100 mm for a ten day period after August, and the degree of progress of the disease.

In the past, Goto, et al²³ pointed out climatic conditions related to the occurrence of this disease during the period from intermediate growth to rice maturity as a mean highest temperature of 26°C for a five day period, a lowest temperature of 22°C, accumulated precipitation of 50 mm, a mean sunshine time of less than eight hours, and a wind

velocity of more than 3 m/sec. They further stated that there was good agreement between the existence of these conditions and the degree of disease occurrence. Fujikawa, et al²¹ pointed out, from results of investigations in the constantly infected areas of Oita Prefecture, such climatic causes as a mean temperature of 24-27°C for a 10 day period, more than seven days of rain during a 10 day period, more than 100 mm of precipitation during a 10 day period, and typhoons. Many of these agree with the results of the investigation of the author.

It is generally known that the occurrence and spread of this disease is closely related to typhoons. In the present research, tests were conducted on the relationship of typhoons, and the results are shown in Chapter XI. However, by the time typhoons reach the Hokuriku District, they are weak, and not like typhoons in the warm areas along the Pacific coast of Japan, and so typhoons in the Hokuriku District do not have a strong impact on the occurrence and spread of this disease. For example, the occurrence process in 1960 (light occurrence year) and the frequency of typhoons as pointed out in Table 82. Furthermore, in the present investigation, the cessation of occurrence progress at the time of high temperatures and aridity during summer (late-July to mid-August), as seen in warm areas, was observed. The author calls this period, period summer cessation.

Section 2. Life Cycle of the Pathogen of Rice Bacterial Leaf Blight in Rice Plants and Irrigation Water

For a two year period from 1956 to 1957 the author made quantitative tests and investigation according to the phage method, on the life cycle of the pathogen on rice plant leaves and in irrigation water during the rice growth period in the affected area in Chikugo, Fukuoka Prefecture. This investigation was conducted for the purpose of the maintenance of bacteria and contamination by bacteria in rice plants and in irrigation water before occurrence. In other words, an investigation of latent infection was made.

This investigation is a part of joint research with Tagami, et al during the author's stay at the Kyushu District Agricultural Experimental Station.^{60,68,69,70,146} The results of the whole investigation together with the results obtained from the investigation continued thereafter will be discussed in detail by Tagami who was in charge of the

project, and will be announced separately.

Therefore, only an outline of the results will be described and only what is related to the tests and investigation made by the author at the Hokuriku District Agricultural Experimental Station in Chapter X and thereafter.

Summary

The occurrence cycle of this disease as observed in 1958-1960 at Takada Municipality, Niigata Prefecture and the life cycle of the pathogen in rice leaves and irrigation water in nurseries and in paddy fields tested by the author at the Kyushu District Agricultural Experimental Station are summarized and shown in Figure 12.

In Figure 12, the life cycle of the pathogen in irrigation water is omitted, but the life cycle of the pathogen on rice leaves and in irrigation water can be shown by a curve related to the time of occurrence and the degree. The present investigation revealed that the timing of occurrence and its degree are considerably influenced by the volume of bacteria contained in seedlings. In the multiplication process of the pathogen in paddy fields, especially on rice leaves, a remarkable increase in the volume of latent bacteria can be observed in the intermediate tillering period and after the young ear formation period. That is, as shown in Figure 12, there are two peak periods in the bacteria curve. The author terms the first peak, at the intermediate tillering period, the first increase period or the first occurrence period, and the second peak, after the young ear formation, the second increase or the second occurrence period. And the trough situated in between these periods, where bacterial force is temporarily at standstill, as the summer cessation period. In addition to this, through this investigation, it was ascertained that the volume of effective bacterial infection per seedling is approximately 500; that the maximum volume of latent bacteria, up to occurrence on rice leaves, is approximately 10^5 ; that there is no certain trend in the life cycle of the pathogen in irrigation water, although it sometimes shows the same process as rice leaves; that natural phages show detection and fluctuation that correspond to the occurrence and the life cycle of the pathogen during the peak tillering period.

These results proved again latent multiplication on rice leaves as clarified by Minakami, *et al*^{79,80,81} by using the concentrated inoculation method in 1955, they also

agreed with the results obtained by the quantitative tests conducted in parallel with this investigation of Sekiya, et al¹¹¹ on the life cycle of the pathogen after inoculation, and also with the result of similar inoculation tests by Kusaba,⁶¹ Mukai, et al^{85,86} by using the streptomycin resistant strain of the pathogen.

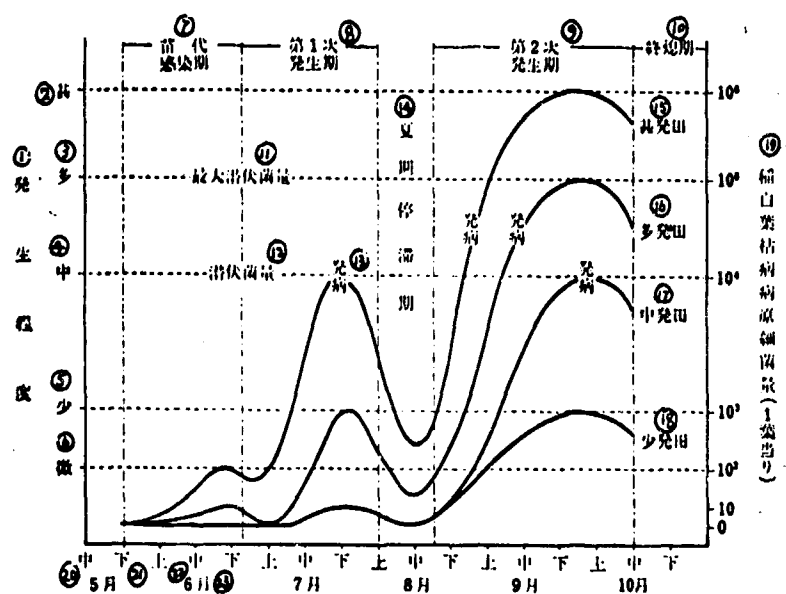


Fig. 12 Occurrence Process of Rice Bacterial Leaf Blight and the Pathogen Life Cycle

[Legend]: 1) Degree of occurrence; 2) Extreme; 3) Much; 4) Medium; 5) Small; 6) Slight; 7) Nursery infection period; 8) First occurrence period; 9) Second occurrence period; 10) Cessation period; 11) Maximum volume of latent bacteria; 12) Volume of latent bacteria; 13) Occurrence; 14) Summer stagnation period; 15) Field with extreme occurrence; 16) Field with much occurrence; 17) Field with medium occurrence; 18) Field with small occurrence; 19) Volume of the pathogen of rice bacterial leaf blight (per leaf); 20) Middle 10 days; 21) Last 10 days; 22) First 10 days; 23) Month.

CHAPTER X. TEST OF PRIMARY INFECTION

In the preceding chapter it was stated that the occurrence of rice bacterial leaf blight is closely related to the volume of infected bacteria in seedlings, to secondary infection by typhoons, and that in the process of bacterial multiplication a temporary decline (the cessation of multiplication) in the summer stagnation period because of high temperature and aridity can be observed. In this experiment, with the exception of secondary infection by typhoons, the following tests were made in order to analyze and examine these phenomena.

Section 1. Relationship Between the Types of Nurseries and Disease Occurrence¹⁷³

The following field test was made at the Hokuriku District Agricultural Experimental Station in 1959 in order to clarify the occurrence process of seedlings raised in different nurseries were transplanted to paddy fields.

1. Test Method

1) Forms of Nurseries, Planted Seeds, and Cultivation

Dry field nursery, wet nursery (these followed the usual method), mixed nursery (water up to nursery surface for about two weeks and water up to $1/3$ height of seedlings), warm mixed nursery (vinyl covering from 19 April to 30 April during which water was below the level of nursery surface), and immersion in deep water after removing covering. Rice varieties: Norin No 29, sowed on 17 April, transplanted on 24 May, Fertilizers: at nursery (per 0.33 a, 225 gr of ammonium sulphate, 150 gr of superphosphate of lime, 110 gr of chlorate of potash), at paddy field (per 10 a.), basic fertilization on 20 May, 37.8 kg of ammonium sulphate, 34.0 kg of superphosphate of lime, 730 kg of manure; additional fertilization, on 25 July, 7.0 kg of ammonium sulphate. Cultivation Density, 30 cm x 18 cm, two stem planting.

2) Area Demarkation and Area

Two areas, one a per area.

3) Investigation of Disease Occurrence

In the investigation conducted on 29 July, 10 August, and 3 September, disease occurrence was investigated on each stem of each area. On 16 September, investigation was made on the degree of occurrence* on check leaves and secondary leaves of 40 stems of one area, by classifying the diseased areas into 10 stages.

2. Test Results and Observations

The results of the tests made by the above method on the relationship between the types of nurseries and disease occurrence are shown in Table 83.

As shown in Table 83, seedlings raised in warm mixed and wet nurseries were infected soonest after transplanting to paddy fields, and the number of infected stems was larger. Seedlings raised in warm mixed nursery showed twice as much percentage of disease occurrence as seedlings raised in a wet nursery. The seedlings raised in mixed nurseries showed a trend toward late occurrence, and occurrence was latest among seedlings raised in dry nurseries. The latter also had the lowest incident of occurrence. The timing and the degree of occurrence at the time of occurrence, as has been stated above, also corresponded to the degree of occurrence at the time of the last investigation of occurrence. The results seem to be due to the difference in water control according to types of nurseries, and to the difference in the susceptibility of seedlings caused by the type of seedling raising. Nasuta, et al⁸⁹ investigated the relationship between the types of nurseries and the disease, and reported that more occurrence in paddy fields was observed among the seedlings raised in warm mixed nurseries than in wet nurseries. They estimated that seedlings raised in warm mixed nurseries would have increased infection. There will be a need for examining such differences not only from the aspect of infection, but also from the aspect of susceptibility.

* Degree of Occurrence % = $0(f_0 + f_1) + (f_2 + f_3) + 2(f_4 + f_5 + f_6) + 3(f_7 + f_8) + 4(f_9 + f_{10}) / 4 \times$ total number of investigated leaves 100. f_n stands for the frequency of percentage of diseased part of each investigated leaf. f_4 shows the total number of leaves which had 40% diseased leaf parts.

Table 83

Relationship Between Types of Nursery and
Occurrence of Rice Bacterial Leaf Blight

Type of Nursery	Area	Percentage of Infected Stems (%)			Degree of Occurrence (%)	
		29 July	10 Aug.	3 Sept.	16 Sept.	Ear Formation
Dry Field Nursery	I	0	0	1	1.4	26 August
	II	0	1	4	1.7	
	Mean	0	1	3	1.6	
Mixed Nursery	I	1	43	57	2.3	" "
	II	0	33	53	1.9	
	Mean	1	38	50	2.1	
Warm Mixed Nursery	I	30	52	47	6.9	25 August
	II	34	56	57	8.1	
	Mean	32	54	52	7.5	
Wet Nursery	I	10	41	44	4.5	26 August
	II	18	55	58	4.8	
	Mean	14	48	51	4.7	

Section 2. The Concentration of Inoculated Bacteria in Seedlings and the Relationship Between the Frequency of Inoculation and Occurrence

In the preceding chapter it was stated that there were some cases in which the degree of infection in nurseries influence the timing of occurrence and its degree after transplanting to paddy fields. This point was already stressed by Kiriū, et al⁵¹ in 1956 from the results of inoculation tests on seedling infection. In 1957, Sekiya, et al¹¹⁸ tested diseased leaf bacteria (as has been previously described) with the inoculation method and recognized that the immersion inoculation method caused a high rate of occurrence in paddy fields even with low bacterial concentration ($n \times 10/\text{ml}$).

The author, in order to determine how much of the bacterial volume in immersion inoculation would be sufficient for seedling infection when cultured bacteria were used, conducted the following inoculation tests in 1962, at the Hokuriku District Agricultural Experimental Station.

1. Relationship Between the Concentration of Inoculum and Occurrence

1) Test Method

Seedling Raising: Warm mixed nursery (Controlled Water Cultivation); variety, Towada; sowed on 23 April and plucked on 28 May.

Inoculum: H6009 strain (pathogenicity, medium, Lyso-type A bacteria) slope cultured on the semi-synthetic agar culture medium for four days at 28°C , was made into a suspension solution with the following concentration, and this was placed in a large photographic vat, and the seedlings were immersed for 24 hours to effect immersion inoculation.

Concentration of Immersion Inoculum: $n \times 10^8$, $n \times 10^7$, $n \times 10^4$, $n \times 10^3$, $n \times 10/\text{ml}$. As a comparison, a city water immersion area was established.

Classification: Five areas, 40 stems each area.

Transplanting to Paddy Fields: On to a general cultivation area of five acres where the Ogyoku variety had already been transplanted on 18 May, immersion inoculated seedlings were transplanted on 29 May according to the plan shown in Figure 14. Cultivation density was 30 cm x 18 cm and one stem transplanting. Sowing and cultivation of rice

plants was generally in accordance with standard cultivation practices at the Hokuriku District Agricultural Experimental Station. The only deviation was that on 13 July 7.5 kg of ammonium sulphate was used as additional fertilization.

Occurrence Investigation: On 22 June, 16 July, 10 August and 3 September, the presence of occurrence was investigated on all the stems in each area.

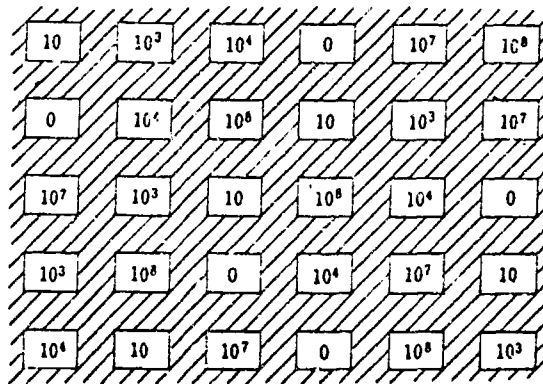


Fig. 14 Plan for Testing the Relationship Between the Volume of Inoculum of Seedlings and Disease Occurrence. Figures within blocks show the concentration of inoculum, and the oblique lined portions show where Ogyoku was cultivated.

Table 84

Relationship Between the Volume of Seedling
Bacteria (concentration of inoculum)
and disease occurrence

Concentration of Inoculum		22 June	16 July	10 Aug.	3 Sept.
City water immersed area	I	0	0	0	1
	II	0	1	5	5
	III	0	0	6	6
	IV	0	1	2	3
	V	0	0	1	2
	Total	0	2	14	17
		(0)	(1)	(7)	(8.5)
$n \times 10^6/\text{ml}$	I	0	2	2	4
	II	0	0	1	1
	III	0	0	2	2
	IV	2	1	8	10
	V	0	2	2	2
	Total	2	5	15	19
		(1)	(2.5)	(7.5)	(9.5)
$n \times 10^3/\text{ml}$	I	1	1	12	14
	II	0	0	2	3
	III	0	1	2	2
	IV	2	2	4	7
	V	2	3	3	3
	Total	5	7	23	29
		(2.5)	(3.5)	(11.5)	(14.5)
$n \times 10^4/\text{ml}$	I	0	1	6	10
	II	0	0	3	3
	III	2	2	7	10
	IV	4	4	5	5
	V	2	2	3	3
	Total	8	9	24	31
		(4)	(4.5)	(12)	(15.5)
$n \times 10^7/\text{ml}$	I	1	2	5	6
	II	0	4	8	10
	III	5	6	10	13
	IV	0	0	2	2
	V	0	1	2	3
	Total	6	13	27	34
		(3)	(6.5)	(13.5)	(17)

	I	2	2	4	4
	II	5	10	13	15
	III	4	5	9	12
	IV	2	16	18	18
	V	4	5	7	8
$n \times 10^8/\text{ml}$		17	33	50	57
	Total	(8.5)	(16.5)	(25)	(28.5)

Note: Number of cultivated stems per plot was 40 stems. Figures in the Table show the number of infected stems. Figures in () equal the percentage of occurrence.

2) Test Results and Observations

The test results are shown in Table 84, and also in Figure 15.

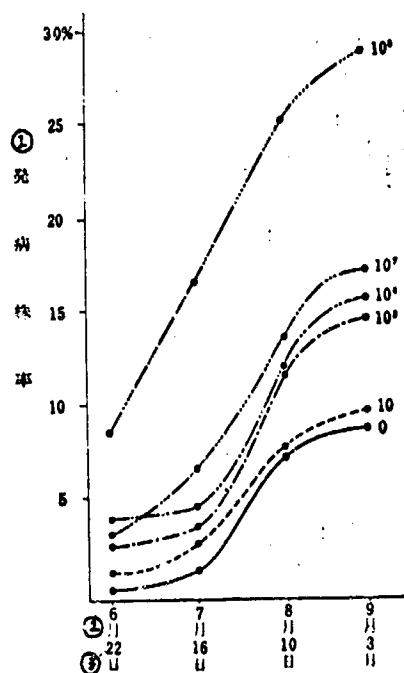


Fig. 15 Relationship Between Volume of Seedling Bacteria and Disease Occurrence

[Legend]: 1) Percentage of infected stems;
2) Month; 3) Day.

The present test was conducted in rice paddies where the resistant Ogyoku variety was planted in order to prevent influencing areas. However, mutual influence among areas was unavoidable, and the test areas were subject to natural infection from surrounding areas. Therefore, the results of this test cannot be strictly termed as differences caused solely by the volume of bacteria contained in seedlings. Nevertheless, even under such conditions, on 22 June, about three weeks after transplanting (24 days), occurrence was observed in each inoculated area with the exception of the city water area set up for a standard comparison. The investigation, on that day, revealed that there were more infected items in proportion to the concentration of inoculum. In the 10^8 concentration of inoculum immersion area, the percentage of infected stems was 8.5%, and 1.0% in the 10^1 immersion area. Thereafter, the monthly investigation revealed a gradual increase in the number of infected stems. However during July occurrence was rare, but it rapidly increased in August. While the influence of secondary infection could be responsible for this, the volume of bacteria contained in seedlings seemed to influence the degree of occurrence in the latter part of paddy field rice growth, since the percentage of occurrence increased correspondingly to the concentration of inoculum. Among the concentration of inoculum, the 10^1 /ml inoculation area showed a similar fluctuation of occurrence as that of the city water immersion area, without too much inoculation influence. In addition to these, occurrence greatly shifted in direct proportion to the concentration of inoculum.

2. Relationship Between the Frequency of Inoculation and Occurrence

As was described in the preceding section, with only one seedling inoculation, even if they were immersed in 10^8 /ml concentrated bacterial solution, occurrence within three weeks of transplanting was only 8.5%, or less than expected. One of the causes was thought to be that in the preceding test, seedlings after inoculation had no bacterial multiplication process, instead they were immediately transplanted to paddy fields.

Therefore, the following test was made in order to investigate the occurrence cycle of inoculated seedlings at different times after their transplantation to paddy fields.

1) Test Method

Seedling Raising: Dry field soil was put into an unglazed pot 20 cm in diameter, and seeds were planted on

28 April. Towada variety. For fertilizer 2 gr each of ammonium sulphate and superphosphate of lime, and 1 gr of chlorate of potash, and 150 gr of manure were used.

Inoculum: H6009 strain slope cultured on the semi-synthetic agar culture medium at 28°C for four days was injected by the following method. Inoculum concentration, approximately 10^7 /ml; inoculation time, 20 hours; inoculation method, immersion inoculation (2/3 of the plants were covered with water).

Composition of Test Areas:

Test Area A,	Immersion inoculation, once,	21 May
" " B	" "	28 "
" " C	" "	4 June
" " D	" "	twice, 21 May
		28 May
" " E	" "	28 May
		4 June
" " F	" "	3 times 21 May
		28 May
		4 June
" " G,	City water immersion, once,	28 May
		(Standard)

Transplanting to Paddy Fields: On 11 June, the foregoing treated seedlings were plucked, and transplanted in two rows for each four rows of Ogyoku in a general Ogyoku cultivation area. 20 stems per one inoculation treatment; one stem planting, repeated twice; cultivation density, 30 cm x 18 cm; and the planting and cultivation followed the standards set by the Hokuriku District Agricultural Experimental Station. The only difference was the addition of ammonium sulphate at the rate of 7.5 kg per 10 acres on 13 July as fertilizer.

Investigation of Occurrence: On 10 June, the presence of disease in seedlings before transplanting was investigated. On 16 July, 2 August, 20 August, and 3 September, the presence of disease was investigated on all the stems in each area.

2) Test Results and Observations

Test results are shown in Table 85, and further shown in Figure 16.

Table 85

Relationship Between Inoculation Time and Frequency of
Seedling and Disease Occurrence in Main Paddy
Fields. (Rate of Occurrence of Stems, %)

Inoculation Method, Frequency	Date of Inoculation	Occurrence of Seedlings	16 July	2 Aug.	20 Aug.	3 Sept.
Immersion Inoculation						
"	once	21 May				
"	"	28 May				
"	"	4 June				
			9.6	13.4	21.6	31.2
			11.1	22.0	20.0	21.8
			1.7	10.5	14.5	13.4
"	twice	21 May.				
"	"	28 May				
"	"	28 May,				
"	"	4 June				
			18.3	26.0	38.0	64.5
			13.9	22.5	31.5	47.0
"	3 times	21 May,				
		28 May,				
		4 June				
			11.4	24.1	37.9	58.5
City water immersed area			-	0.5	5.0	11.0
						12.5

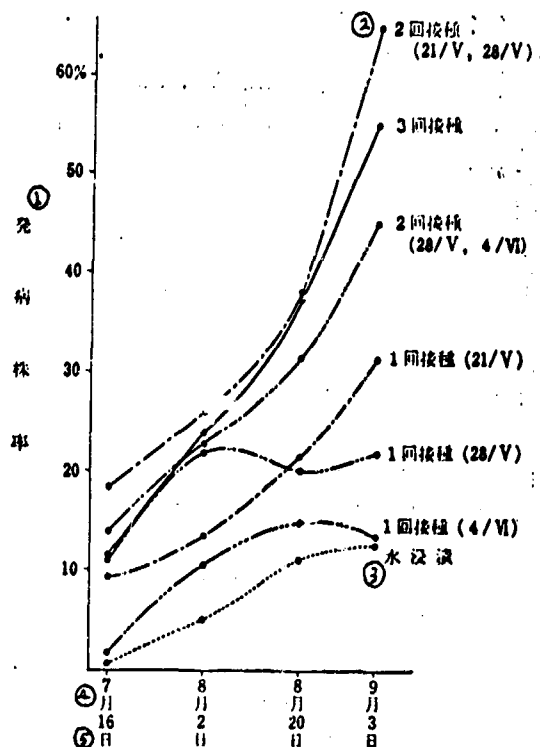


Fig. 16 Relationship Between Seedling Inoculation Time and Frequency and Occurrence in Paddy Fields

[Legend]: 1) Percentage of infected stems; 2) 2 inoculations (21 May, 28 May); 3) Water immersion; 4) Month; 5) Day.

No disease occurrence was observed before transplanting in any inoculation areas. It seemed that even though there was bacterial multiplication, bacteria was insufficient to cause occurrence. In this test, H6009 strain preserved at the Hokuriku District Agricultural Experimental Station was selected as the test sample. Its pathogenicity was less than medium, and this might have been the reason for no occurrence of the disease among seedlings even 20 days after inoculation. However investigation on 16 July, about one month after transplanting, showed considerable occurrence in each area. The rates of occurrence in the seedling areas with two or three inoculations was 18% one month after transplanting, and 65% at the last investigation.

Seedlings which were inoculated earlier showed more occurrence than those late inoculated ones. Especially, the areas which were inoculated on 21 May and 28 May showed many occurrences regardless of the frequency of inoculation. It was estimated that as a result of sufficient time for bacterial multiplication, which was possible because of immersion inoculation during the intermediate period at nurseries, bacterial multiplication progressed without too much delay, and occurrence broke out in most stems.

3. Summary

The results of tests made on the relationship between the concentration and frequency of inoculum on seedlings and the occurrence after transplanting to paddy fields are summarized as follows:

1) Seedlings immersed in bacterial solutions of 5-stage concentrations (10^8 , 10^7 , 10^4 , 10^3 , and 10^1 /ml) for 24 hours showed occurrence after transplanting, three weeks after inoculation, with the exception of the city water immersion area, and the 10^8 /ml inoculation area. The degree of occurrence corresponded to the concentration of inoculum. Thus in the 10^8 /ml inoculation area, occurrence was 8.5%, the highest percentage. The increase of occurrences thereafter, also showed a trend toward more occurrence in areas of high concentration of inoculum or the areas with more bacteria.

2) As to the relationship between the time and frequency of immersion inoculation in nurseries, more occurrence after transplanting to paddy fields was observed in those seedlings with more frequent inoculations and earlier inoculations in the latter half period in the nurseries. It was ascertained that the frequency of infection in nurseries and the presence of bacterial multiplication in seedlings had a strong influence over the occurrence in paddy fields.

Section 3. Relationship Between the Multiplication of the Pathogen of Rice Bacterial Leaf Blight on Rice Leaves and Temperature

It has been stated that bacterial multiplication on rice leaves ceases during the high temperature period in summer. Tests were made in 1960, at the Hokuriku District Agricultural Experimental Station, in order to determine the suppression of bacterial multiplication by high temperature and aridness.

1. Test Method

1) Cultivation of Test Rice

Dry field soil was put into 15 x 25 x 15 cm tin boxes. Fertilization consisted of 3 gr of ammonium phosphate and superphosphate of lime and 1 gr of chlorate of potash per box. 30 grams of seeds were planted per box on 15 July.

2) Inoculation Method

The H5838 strain, slope cultured on the semi-synthetic agar culture medium at 28°C for four days, bacterial solution of the two stage bacterial concentration of $n \times 10^5$ and $n \times 10^7$ was used. At 6 pm, 5 September, 6 ml of this was spray inoculated into each box.

3) Composition of Test Areas

For three days after inoculation, the boxes were left outdoors. Thereafter, the boxes were moved to the designated places as shown in Table 86.

Table 86

Composition of Test Areas

Test Area	Concentration of Inoculum	Remarks
High temperature arid area	2×10^5 , 10^7 /ml	Glasshouse with windows open during daytime (highest temperature..average 35°C, 10 am. humidity... 61.5%)
Outdoors	2×10^5 , 10^7 /ml	Highest temperature...26.1%, humidity...83.5% (mean figures at 10 a.m.
Standard non-inoculation area		Left isolated in fields.

4) Quantitative Test for Bacteria

One box of rice plants was used for test samples each time. 20 leaves were taken and placed in a 500 ml capacity flask and 100 ml of sterile water was added and shaken 300 times. The bacterial suspension solution thus obtained was used for the quantitative test for bacteria in accordance with the third method, Figure 9, for each period shown in Table 88.

5) Investigation of Occurrence, Measurement of Temperature and Humidity

In the period of first infection and on 29 September, the percentage of infected leaves and the percentage of diseased area were investigated. During the test period, temperature and humidity were checked each day at 10 a.m., and the highest and lowest temperatures were also measured.

Table 87

Life Cycle of Bacteria in the High-temperature-arid Area and the Outdoor Area (Volume of Bacteria per Leaf)

Elapse Time	10 ⁷ /ml Inoculation Area		10 ⁵ /ml Inoculation Area		Standard non-inoculation area
	Glass-house	Outdoors	Glass-house	Outdoors	
20 hours		424		25	0
44		52		113	6
72		155		41	0
96	920	607	25	50	0
120	465	14,525	51	575	13
168	357	95,700	0	2,025	3
216	675	197,250*	10	8,500	6
11 day	1,682	1,082,000	28	51,375	95
15 day	390	N	49	781,600*	0
% of infected leaves	0	30.3	0	2.7	0
Area of diseased area**cm ²	0	11	0	9	0

Note: * First infection, ** Maximum diseased area.

Table 88

Climate During Inoculation Tests

Date	Highest Temperature (°C)		Lowest Temperature (°C)		Temperature at 10 a.m.		Humidity (%)		Weather
	Outdoors	Glass-house	Outdoors	Glass-house	Outdoors	Glass-house	Outdoors	Glass-house	
6 Sept	26.6		18.0		24.3		82		
7	25.3	34.0	20.5		22.6		89		73
8	27.0	33.5	19.0	26.5	26.0	32.0	79		54
9	24.0	32.5	20.6	24.5	23.7	29.0	83		59
10	26.0	37.0	20.5	25.0	24.0	30.0	82		56
12	26.7	37.0	19.3	24.0	21.4	26.0	92		63
14	30.4	38.0	20.2	25.0	27.9	34.0	74		52
16	25.1	36.0	19.0	22.5	24.2	33.0	79		73
20	25.3	37.0	19.8	25.5	21.6	32.0	92		66
26	24.9	31.0	18.7	24.0	21.1	31.0	-		58
Mean	26.1	34.9	19.6	25.0	23.7	31.0	83		60

2. Test Results and Observations

The volume of bacteria on leaves in outdoor conditions and in the glasshouse, occurrence, and the measurement of temperature and humidity during the test period are shown in Tables 87 and 88, and Figure 17.

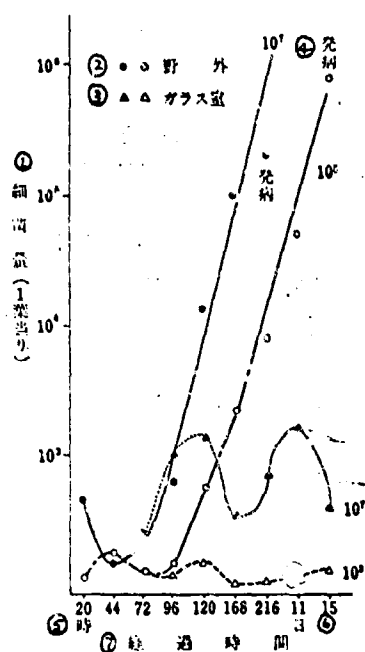


Fig. 17 Life Cycle of the Pathogen of Rice Bacterial Leaf Blight Under High Temperature and Arid Conditions

[Legend]: 1) Bacteria per leaf; 2) Outdoors; 3) Glasshouse; 4) Occurrence; 5) Time; 6) Day; 7) Elapse of time.

As shown in Table 87 and Figure 17, bacterial multiplication was observed four days (96 hours) after inoculation in the outdoor area with a 10^7 /ml bacterial concentration. The bacteria showed rapid increase thereafter. In nine days (216 hours), there were 10^5 bacteria per leaf, and the immersed diseased area along leaf edges could be observable on some leaves with the naked eye. Against this, in the case of the glasshouse section of the high temperature and arid area transferred three days after inoculation, there was only a slight bacteria increase after 96 hours. Bacteria increase ceased thereafter and tended to decrease.

15 days after inoculation, just about the same volume of bacteria was detected as that detected immediately after inoculation.

In the case of the outdoor area, inoculated with a 10^5 /ml bacteria concentration, the period of bacterial multiplication was delayed, 210 hours after inoculation. Otherwise, it showed the same process as in the case of the 10^7 /ml inoculation area. However in the case of the glasshouse area, almost no increase, bacteria, or occurrence were observed.

As shown in Table 88, the extraordinary high temperature and aridity in the glasshouse (highest temperature, 40°C and 52% humidity) seemed to have suppressed bacterial multiplication. Mukai, et al⁸⁶ thought the expansion of diseased spots in the needle inoculation was in proportion to the rise of temperature, to 31°C . However, in the test made by the author, by spray inoculation, the temperature was unordinarily high and the air was dry. Consequently, the cause of the temporary bacteria decrease on rice leaves under high temperature and aridity during summer has been partially explained.

3. Summary

The multiplication of the pathogen of rice bacterial leaf blight (on rice leaves) is clearly suppressed when the average highest temperature is 34.9°C , and a low humidity of 60%.

CHAPTER XI. TEST FOR SECONDARY INFECTION¹⁵¹

It has often been observed that the occurrence and spread of rice bacterial leaf blight is severe after typhoons. This seems to be because either the winds cause lesions on rice leaves or the bacteria on infected leaves are dispersed and spread to uninfected stems or rice paddies. This test was made for secondary infection by wind dissemination in 1956 at the Kyushu District Agricultural Experimental Station.

Section 1. Test on Distance of Wind Dissemination

1. Test Method

Plots were assigned in spiral form at certain intervals, as shown in Figure 18, in a large field area void of intermediate host plants (such as dry field rice plants) of the pathogen of rice bacterial leaf blight and without the hazard of immersion. Then the dry field Norin type glutinous No 1 rice was planted on 30 June and cultivated in a circular 10 meter in diameter field. As for the source of infection, dry field Norin type glutinous No 1 was planted in two rows in concentric circles 10 meters in diameter. 20 days after planting (20 July), pathogens were developed (affected leaves of paddy rice) through spray inoculation. This was then disseminated by the wind.

The distances from investigation points to the source of inoculation were 0.5 m, 1 m, 2 m, 4 m, 8 m, 16 m, 32 m, and 64 m. The distances among these points were arranged in such a way as to increase the distance from the source of infection. Detection and quantitative tests, in accordance with the first method, Figure 9, were conducted by collecting 10-20 rice leaves at each point at certain intervals of time after inoculation.

2. Test Results and Observations

The volume of bacteria on rice leaves at inoculation points and plots from wind dissemination and disease

occurrence are shown in Table 89. The mean dissemination volume at each plot, is shown in Figures 18-19. The frequency of winds during the test period by month and wind velocity are shown in Table 90.

Table 89

Test Results on Distance of Wind Dissemination
of the Pathogen of Rice Bacterial Leaf
Blight (Volume of Bacteria per Leaf)

① 接種源か らの距離		調査月 日②						③発病調査		
7月22日		7月31日	8月10日	8月24日	9月4日	9月14日	平均	9月4日	10月16日	
⑥接種 地点	A	2,000	280×10 ⁴	1×10 ⁵	60×10 ⁴	2.5×10 ⁵	8.5×10 ⁴		+	+
	B	"	16×10 ⁴	7×10 ⁵	5×10 ⁴	2×10 ⁴	5×10 ⁴		+	+
50cm			590	29	10,244	1,094	1,295	2,650	+	+
1m			415	0	6,796	1,619	1,674	2,100	+	+
2m			174	0	4,964	1,111	2,053	1,660	+	-
4m			110	0	8,747	845	1,106	2,160	-	-
6m			181	0	1,377	0	0	310	-	-
16m			116	0	1,997	1,447	2,109	1,130	-	-
32m			0	0	475	1,784	773	600	-	-
64m			0	0	3,726	1,288	0	1,000	-	-

Note: Inoculation on 29 July (bacteria of diseased leaves) by spraying. About 10 day after occurrence was observed.

[Legend]: 1) Distance from the source of inoculation; 2) Date of investigation; 3) Month; 4) Day; 5) Investigation of occurrence; 6) Inoculation point.

Table 90

Frequency of the Most Frequent Wind During the
Test Period (20 July-30 September 1956)

③ 風向	①月		7月			8月			9月			7~9月計②			⑥ 合計
	② 風速	④	1~	3~	5m	1~	3~	5m	1~	3~	5m	1~	3.0~	5m	
			2.9m	4.9m	④以上	2.9m	4.9m	以上	2.9m	4.9m	以上	2.9m	4.9m	以上	
N			1	1		1	6	1	2	2	1	4	9	2	15
NE							2						2		2
E				1		1	3			1	2	1	5	2	8
SE				1			1	1	1	3		1	5		7
S				1	1	3	3	3		3	2	3	7	6	16
SW				3		2		1	1	2	1	3	5	2	10
W			1						1	1		2	1		3
NW			1			1				4	2	2	4	2	8

Note: With the exception of windless days, only those days with more than 1 m wind velocity and wind blowing for over 10 hours are shown.

[Legend]: 1) Month; 2) Wind velocity; 3) Wind direction; 4) More than; 5) Monthly total; 6) Total.

Fluctuation in the volume of bacteria at the inoculation points, as the source of infection, are shown in Table 89. At the time of investigation, 31 July, about 10 days after inoculation, 10^{4-6} of the bacteria per leaf were detected and disease occurrence was observed. However there was unusual dryness from mid-July to early August, and this drought affected the growth of rice plants. Thus the volume of bacteria was somewhat decreased, and occurrence almost ceased. This is the bacterial multiplication process on rice leaves at the time of drought, and it may be regarded as the life cycle of bacteria when additional infection from other sources, after inoculation, are lacking. At any rate, wind dissemination was not a good source because of the dryness.

Bacterial dissemination at the plots is shown in Table 89. Already 10 days after inoculation, bacteria were detected from the 0.5 m area to the 16 m area, but no detection was made in the 32 m and 64 m areas. A trend toward a decrease in the volume of disseminated bacteria in proportion to distance was observed. However, at the time of investigation on 10 August, the decrease of bacteria was

① 大豆畑

② 2.101

③ 1.660

④ 2.162

⑤ 312

⑥ 1,134

⑦ 606

32 m

64 m

2,650

1 m

2 m

4 m

8 m

16 m

Ino.

N

② ○ の内附数字は地点番号
 ③ ○ の外附数字は飛散距離
 ④ ● は発病 ○ は無発病 ● ±

[Legend]: 1) Soy bean field; 2) Figures inside are numerical numbers of points; 3) Figures outside are the volume of bacteria disseminated; 4) ⊙ occurrence; 5) ⊖ non-occurrence.

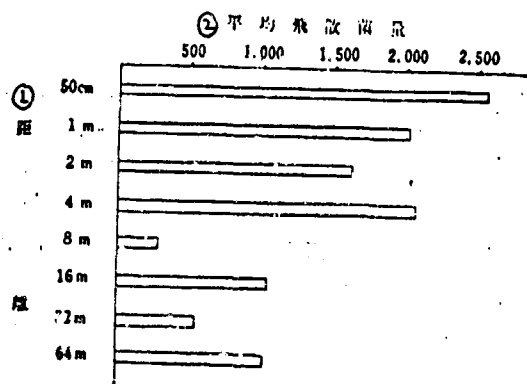


Fig. 19 Volume of Disseminated Bacteria by Distance with a Wind Velocity of 28m/sec. (volume of bacteria per leaf)

[Legend]: 1) Mean volume of Disseminated bacteria; 2) Distance.

It is noteworthy that the order of intensity was in the order of distances. Particularly at the points of 8 m, 16 m, and 32 m, rather than at the point of 64 m, the volume of bacteria was less. The detection of the volume of bacteria that are not in direct proportion seems to be related to the most frequent winds and wind velocity up to that time. That is, as shown in Table 90, there was almost no wind that blew from the direction of the source of inoculation toward these points (N-NE-E). In other words, dissemination was influenced by the wind blowing mostly toward the 64 m point, the S-SW wind, and the wind direction of typhoon No 9 (SE-S-SW), as shown in the test results on the direction of wind dissemination by the typhoon. Thus, under the condition of such winds, the distance of wind dissemination seems to be as far as 64 m.

Investigation on 4 September and thereafter showed a decrease in the general volume of bacteria at each point. Because a similar trend was observed at the source of inoculation, it seems that the decrease was due to the adverse environment for bacterial multiplication.

In this test, the relationship between the distance from the source of inoculation, the volume of disseminated bacteria, and the strength of the wind were examined. However, accurate observation was impossible because the plots

for wind dissemination were not in the same direction. The mean totals of the volume of disseminated bacteria (including auto-generating bacteria) of each investigation are shown in Figure 19. From this it seems that the shorter the distance, the larger the volume of bacterial dissemination. However, in this test, the volumes of bacteria at the 8 m and 32 m areas were not in direct proportion to distance, but this seems to have been caused by the direction of wind dissemination.

Although there were differences in the volume of disseminated bacteria, each point seems to have had bacterial infection by wind. However, at the time of investigation on 5 September (ear filling period), identification of affected leaves was difficult because of leaf damage and brown leaves because of several typhoons. Disease occurrence was positively observed only in the 0.5 m and 1 m areas, and the results were \pm in the 2 m and 4 m areas. Therefore, according to these tests, the margin of distance which led to occurrence by wind dissemination and bacterial multiplication was within 4 m.

3. Summary

The results of tests with dry field rice for the distance of wind dissemination reveals that the pathogen of rice bacterial leaf blight was dispersed up to 64 m by wind dissemination with a wind velocity 28 m/sec; that more bacteria were dispersed to closer distances; and that the direction of dissemination and the direction of wind at the time were in a parallel relationship. The effective distance range in which disease occurred due to multiplication of disseminated bacteria on rice plants was 4 m. This does not imply, however, that the importance of wind dissemination should be ignored. In actuality, it results in repeated multiplication and bacterial dissemination. Thus the dissemination seems to cover a wide area.

Section 2. Test on the Distance of Bacterial Dissemination by Typhoons and Directions

1. Test Method

1) Test for Dissemination Distance

By using Nos 12 and 15 typhoons on 9 and 26 September 1959, as in the previous test, as shown in Figure 20, investigation points were set up at certain intervals in

the three directions of Northwest (A row), North (C row) and Northeast (B row) in a large dry field area that had no immersion hazard and without intermediate host plants of this pathogen. Poles were set up at these points and sterilized cheese-cloth slides were inserted on their tips to catch the bacteria dispersed from 24 pots of diseased, through inoculation rice plants which served as the centers of wind dissemination. The first method of Figure 9 was used. The distances from the source of inoculation were 3.75 m, 7.5 m, 15 m, 30 m, and 60 m, respectively. And the time the glass slides inserted in the poles were exposed to the bacterial dispersion by typhoons was as follows:

Experiment at the time of Typhoon No 12: Nine hours from 11:10 p.m., 9 September to 8:00 a.m., 10 September.

Experiment at the time of Typhoon No 15: 12 hours from 8:00 p.m., 26 September to 8:00 a.m., 27 September.

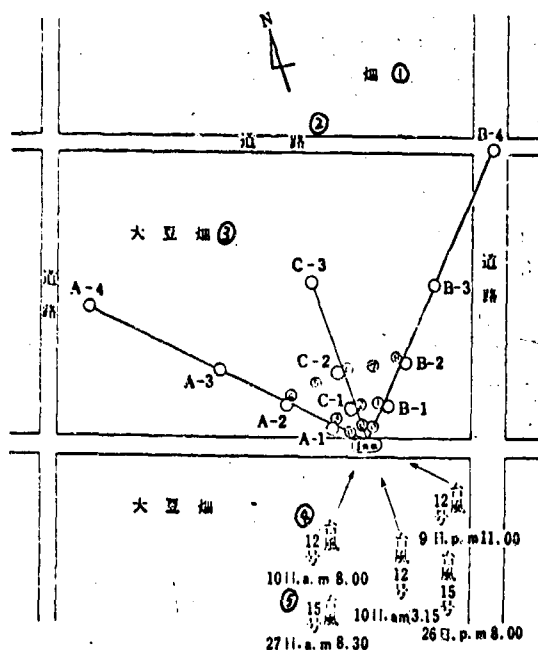


Fig. 20 Sketch of Test for Dispersion Distance and Direction of the Pathogen of Rice Bacterial Leaf Blight by Typhoons
24 inoculated infected rice pots were used.

Legend: 1) Dry field; 2) Road; 3) Soy bean field; 4) Typhoon No 12; 5) Day.

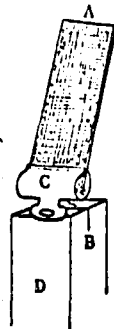


Fig. 21 Cheese-cloth Slide Catcher

Legend: A) Cheese-cloth slide; B) Slot; C) Clip; D) Pole.

2) Test for the Direction of Wind Dissemination

At the time of typhoon No 12, for the test on the direction of wind dissemination, eight investigation points as shown in Figure 20 were established and poles were set up at these points. At the time the wind began to blow from the inoculation source pots toward the center of the fan-shape dispersion, the sterile cheese-cloth slides were inserted in poles and exposed to the wind. After they caught the bacteria, they were immediately removed from the poles, before the typhoon changed direction, and placed in large test tubes. The quantitative test for bacteria was then made in accordance with the first method of Figure 9. The time exposed to wind was about four hours, from 3:15 a.m., 10 September to 7:30 a.m.

The Cheese-cloth slides used for the test were wet sterilized just before test as they were inserted in the clips. As shown in Figure 21, the clips were inserted in the slots at the tip of poles. After they were exposed to the wind and bacteria, they were removed from clips, and were plunged into separately prepared large test tubes and shaken. The bacteria, thus suspended in sterile water, was quantitatively tested. While this cheese-cloth method cannot be used to detect occurrence due to wind dissemination, as in the case of rice plants, they have the advantage of catching an actual number of dispersed bacteria.

2. Test Results and Observations

The results of the quantitative test by the method described above on the volume of dispersed bacteria by distance and direction are shown in Tables 91 and 92, and Figures 22 and 23.

As shown in Table 91, the dispersion of bacteria by typhoons was certain up to 30 m with a wind velocity of 5.3 m -22 m/sec, and no bacteria were detected at 60 m. The shorter the distance, the larger the volume of dispersed bacteria. As shown in the test results, at the time of typhoon No 12, high velocity winds caused more dispersion volume. As shown in Table 92 and Figure 22, when the direction of wind inclined 45° , the volume of dispersed bacteria was reduced about two-thirds.

Table 91

Test Results on Distance of Wind
Dissemination by Typhoons

Point No.	Distance	Volume of Dispersed Bacteria		
		Typhoon 12	Typhoon 15	
Row A	0	3.75 m	-	1,166
	1	7.5	10,317	518
	2	15.0	8,727	251
	3	30.0	5,691	191
	4	60.0	0	0
Row C	0	3.75	7,650	-
	1	7.5	13,920	-
	2	15.0	15,320	-
	3	30.0	7,536	-
Row B	0	3.75	-	795
	1	7.5	10,287	-
	2	15.0	8,079	-
	3	30.0	729	-
	4	60.0	0	-

Note: Figures in table show the volume of bacteria per slide. (Surface area of one slide is 20 cm²)

Table 92

Test Results on Direction of Wind
Dissemination by Typhoons

Point No.	Distance	Direction	Volume of Dispersed Bacteria
1	7.5 m	NE	5,673
2	"	N	12,759
3	"	NW	9,003
4	15.0	NW	8,283
5	15.0	NNW	3,421
6	"	N	13,269
7	"	NNE	5,469
8	"	NE	7,287

Note: Figures in table show the volume of bacteria per slide. (Surface area of one slide is 20 cm²)

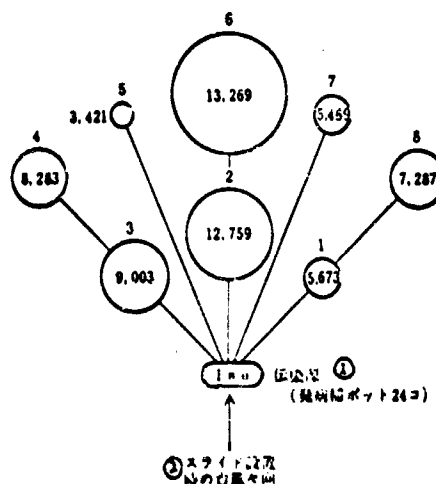


Fig. 22 Test Results on Direction of Wind
Dissemination by Typhoons

[Legend]: 1) Inoculum (24 pots of infected rice); 2) Direction of typhoon.

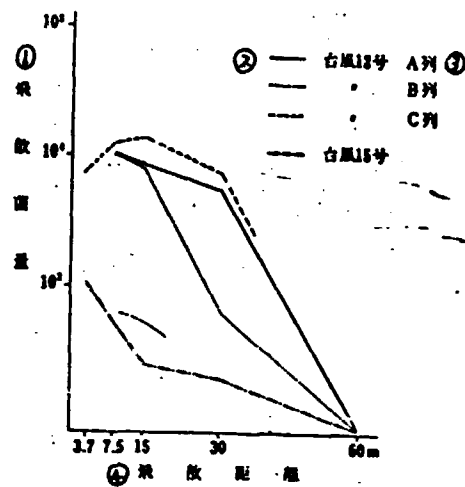


Fig. 23 Test Results on Distance of Wind Dissemination by Typhoons

[Legend]: 1) Volume of dispersed bacteria;
 2) Typhoon No; 3) Row; 4) Distance of dispersion.

Table 93

Investigation of the 1956 Rice Growing Period Typhoons

Item	Typhoon No. 9	Typhoon No. 12	Typhoon No. 15
Date	16-17 August	9-10 September	26-27 September
Lowest atmospheric pressure	974.9mb (5:02 a.m., 17 August)	985mb (2:15 a.m., 10 September)	1018.6mb (1:40 a.m., 26 September)
Maximum wind velocity	28 m/sec (5:10 a.m., 17 Aug-5:20 a.m.)	22 m/sec. (4:00 a.m. 10 Sept 6:00 a.m.)	5.3 m/sec (2:00 a.m., 26 Sept)
Daily Precipitation	83.1 mm (9:00 a.m., 16 Aug-9:00 a.m., 17 August)	23.7 mm (9:00 a.m. 9 Sept-9:00 a.m., 10 September)	85.5 mm (25-26 Sept.) 12.9 mm (26-27 Sept.)
Maximum hourly precipitation	29.0 mm (3:50-4:50 a.m., 17 August)	7.0 mm (4:00-5:00 a.m. 9 September)	14 mm (7:00-8:00 a.m. 25 September)
Maximum temperature	28.8°C (16) 28.5°C (17)	32.7°C (9 Sept.) 27.1°C (10 September)	30.7°C (25 September) 20.8°C (26 September)
Minimum temperature	22.6°C (16 August) 22.7°C (17 August)	22.3°C	19.1°C (25 September) 19.0°C (26 September)
Most frequent wind direction	SE-S-SSW	SE-S-SW	SE-SWW-WNW

Section 3. Test on Effect of Water Drops in Wind Dissemination

In actual wind dissemination, wind as well as the dispersion of raindrops take part. In this test, the volume of bacterial dispersion when only wind was directed at the inoculation infected stems (source of wind dissemination) by the aforementioned slide method with a mist machine, and the dispersion of bacteria by spraying water were quantitatively tested and compared according to the first method of Figure 9.

1. Test Method

Rice planted in 1/2,000 pots (variety, Jugoku) are spray inoculated several times beforehand, led to occurrence, and used as the source of infection. There were two pots of inoculation infected stems, and two stems of each were cultivated per pot.

This infection method was in accordance with the cheese-cloth slide method as described in the preceding section, and the distance to the diseased pot was set at 2 m. The Kyoritsu Mist Machine was used. The wind velocity when the wind was blown was 37 m/sec at the nozzle and infected stems, and the wind velocity near the slide was 2 m/sec. The time of wind exposure was 30 minutes, and the wind channel was marked by vinyl to make the effect of the wind high.

2. Test Results and Observations

The test results on the effect of water drops at the time of wind infection are shown in Table 94.

Table 94

Test on the Effect of Water Drops
in Wind Infection

Test Area	Volume of Dispersed Bacteria			Dispersed phage
	I	II	Mean	
Only wind blown	174	666	420	0
Water drops and wind blown	1,982	1,584	1,780	10,350

Note: Figures show the volume of dispersed bacteria per slide.

As shown in Table 94, bacterial dispersion from infected stems was clearly observable. When the case of only wind blowing was compared with the case of wind with water drops, in the former, the volume of dispersed bacteria was small. It was estimated therefore that in fields, in general, the degree of wind infection varied according to the presence of rain drops.

Section 4. General Summary

- 1) With a maximum wind velocity of 28 m/sec, the bacteria from affected rice plants were dispersed as far as 64 m.
- 2) The distance of occurrence of the dispersed bacteria was less than 4 m.
- 3) The dispersion of bacteria agreed with the direction of wind.
- 4) More volume of bacteria was dispersed by wind accompanied by rain. This was confirmed by comparative tests with dry winds.

CHAPTER XII. RELATIONSHIP BETWEEN THE LIFE CYCLE OF THE PATHOGEN PHAGE OF RICE BACTERIAL LEAF BLIGHT IN IRRIGATION WATER AND DISEASE OCCURRENCE

As was described in Chapter IX, in the process of the quantitative analysis of the multiplication of the pathogen of rice bacterial leaf blight during the rice growth period conducted by the author in conjunction with Tagami⁶⁸, the phages in the test sample concurrently with the quantitative test for bacteria, were measured. The results revealed that in rice paddies where this disease frequently occurs much phage was detected in the water of rice paddies, and the increase of phages before occurrence in rice plants was observed.

Tagami, et al⁶⁸⁻⁷⁰ and the author noted the detection of phages in the early period, in the water of rice paddies, and examined the quantitative analysis of phages existing mainly in irrigation water and their relationship to the occurrence of this disease. Tagami, et al^{120-122, 124, 125} already made quantitative tests of the water of nursery beds and paddy fields, and observed that there was a close relationship between the volume and the degree of occurrence in paddy field.

From the same viewpoint the author made investigations in 1958 and thereafter at the Hokuriku District Agricultural Experimental Station on quantitative phage changes in irrigation water when the sample phages were collected from irrigation water and rivers in infected areas and the process of occurrence in the vicinity. Concurrently, he examined, over a large area whether preliminary diagnosis of the occurrence of this disease was possible.

Section 1. Quantitative of Phage Method 121

The water in nursery beds or paddy fields and the irrigation water from ditches, channels, reservoirs, and rivers was scooped into sterile glass containers or polyethylene containers. A certain volume (1-0.01 ml) was mixed with about 2 ml of the concentrated suspension

solution of this pathogen ($= 10^9/\text{ml}$), and 5 ml of the melted semi-synthetic agar culture medium (which had been separately melted with hot water and placed in a thermostat tank at about 60°C) cooled to about 50°C , shaken, and immediately placed in a bowl to be made into plaques. The measurement of plaques was made after they were preserved in a thermostat at 28°C for eight to ten hours, and this was repeated two or three times. Then, the volume of phage per 1 ml of test sample (irrigation water) was computed. As for bacteria used for the quantitative method, strains with different phage affinity were used depending on the purpose of the tests. All of them were slope cultured on the semi-synthetic agar culture medium at 28°C for four to five days, and from these, bacteria suspension solution was produced. As the phage indicator strains, Shijon strain (lyso-type A), Benikonaya strain (Lyso-type B), H5925 (lyso-type D), and H5913 (lyso-type E) were used.

Section 2. Relationship Between the Volume of Phages in the Water of Nurseries and Paddy Fields and Disease Occurrence

From 1959 to 1960, for two years, the following investigation took place in the vicinity of Takada Municipality, Niigata Prefecture in order to examine the relationship between the volume of phages in the water of nursery beds and paddy fields, and the degree of occurrence in paddy fields.

1. 1959 Test

1) Test Method

a) Investigated nursery beds: 21 patches of nurseries in Takada Municipality where susceptible varieties Norin No 29, Sanin No 52, Echiei, and Kinnanpu were arbitrarily selected.

b) Paddy fields: Investigation was made of the 41 patches of seedlings transplanted from the foregoing nurseries to paddy fields.

c) Quantitative test for phage: By following the method described in the preceding section, the water from the nurseries was scooped up on 29 April-1 May, 19 to 23 May, and from paddy fields on 19 to 21 June and 13 to 17 July, and the quantitative test for phages per 1 ml of sample water was made.

d) Investigation of disease occurrence in paddy fields: From 10 to 16 September, the degree of occurrence, which was classified into extreme, much, medium, small, and slight stages in accordance with the standards for the preliminary observation of disease occurrence,* was surveyed for each patch. Since it was difficult to find paddy fields with only one variety of rice, fields with several varieties as mentioned above as susceptible varieties were selected.

2) Test Results

The volume of phage in the water of nurseries was investigated in 1959, as shown in Table 95. The volume of phages in the water of paddy fields where seedlings were transplanted and the degree of occurrence corresponding to these are shown in Table 96.

Table 95

Volume of Phage in the Surface Water of Nurseries
(1959, Takada Municipality, Niigata Prefecture)

Nursery Number	29 April-1 May	20 May-23 May	Total Phage
1	3	26	29
2	0	24	14
3	0	8	8
4	1	4	5
5	0	0	0
6	0	0	0
7	0	0	0
8	2	0	2
9	1	0	1
10	0	0	0
11	0	0	3
12	0	15	15
13	1	0	1
14	0	0	0
15	0	4	4
16	0	1	1
17	0	0	0
18	2	2	4
19	2	2	4
20	0	0	0
21	0	0	0

* Development Bureau, Ministry of Agriculture and Forestry (1958): Principles of the Execution of Preliminary Diagnosis of Insect Diseases, page 15.

Table 96

Relation Between the Volume of Phage in the Surface
Water in Nurseries and Paddy Fields and Disease
Occurrence (1959, Various Points, Takada
Municipality, Niigata Prefecture.)

Nursery Number	Total Phage Measured at Nursery	Paddy Field Number	Variety	Volume of Phages in		Degree of Occurrence in Paddy Field	Discovery of First Occurrence
				Paddy Field			
				19-21 June	13-17 July		
/ml							
1	29	1	Kinnanpu	1,119	11,383	Extreme	26 June
		2	Norin No. 29	449	12,236	Much	" "
		3	Kinnanpu	2,156	23,666	Extreme	" "
2	14	4	Kinnanpu	424	17,269	Extreme	30 June
3	8	5	Echiel	97	1,063	Medium	28 July
		6	Kinnanpu	606	4,038	Much	" "
4	5	7	Sanin No. 52	12	4,638	Medium	28 July
		8	Echiel	16	5,253	"	" "
5	0	9	Norin No. 29	0	125	Slight	16 August
		10	Kinnanpu	3	1,394	Small	" "
		11	Sanin No. 52	0	73	Slight	" "
6	0	12	Norin No. 29	72	6,451	Medium	28 July

7	13	Sanin No. 52	11	1,352	Medium	16 August
	14	"	2	250	"	"
	15	Kinnanpu	7	2,446	Much	"
8	16	Echiel	10	364	Much	16 August
	17	Norin No. 29	13	926	Medium	"
	18	"	2	1,062	"	"
9	19	Kinnanpu	21	3,815	Much	28 July
	20	Norin No. 29	17	350	Medium	16 August
10	21	Echiel	0	142	Medium	16 August
	22	Norin No. 29	0	52	Small	"
11	23	Sanin No. 52	15	3,824	Much	28 July
	24	Norin No. 29	11	730	Medium	16 August
	25	Hokuriku No. 52	13	505	"	"
12	26	Norin No. 29	348	22,610	Much	2 July
	27	Nihonkai	419	13,490	Much	"
13	28	Echiel	316	9,480	Medium	2 July
14	29a	Norin No. 29	12	240	Small	16 August a
	29b	Hokuriku No. 52	16	720	"	b
15	30	Shin No. 7	43	4,580	Much	28 July

16	1	31	Hokuriku No. 52	0	110	Medium	16 August
17	0	32	Sarin No. 52	13	972	Much	16 August
		33	Norin No. 29	32	206	Medium	"
18	4	34	Echiel	90	13,170	Much	2 July
19	4	35	Nihonkai	225	8,720	Much	2 July
20	0	36	Norin No. 29	111	3,460	Medium	28 July
		37	"	10	1,610	Small	16 August
		38	Echiel	17	3,260	Small	"
21	0	39	Kirnanpu	162	20,130	Extreme	2 July
		40	Koganemochi	34	9,820	Much	19 July
		41	Nihonkai	71	5,920	Much	"

Table 97

Comparison of Volumes of Phages at the Time
of Non-occurrence and Occurrence

Paddy Field Number	No Occurrence 19-21 June	Occurrence 2 July
1	1,119	13,200
2	449	2,860
3	2,156	18,490
4	424	19,950
26	348	4,750
27	419	11,990
28	315	8,060
34	90	9,900
35	225	10,200
39	162	7,960

3) Results and Observations

a) Occurrence in the Investigated Area in General

In late June 1959, (intermediate tillering period) four weeks after transplanting to paddy fields, several patches of infected fields manifesting acute wilting symptoms at several investigation points were observed. There was heavy rain on 1-2 July (114.2 mm), and on 11 July (160.2 mm). Thus by mid and late July, (peak tillering period), occurrence was observed throughout almost the whole area. The infected area and degree of this year, were record breaking ones for the district.

b) Phages in Nurseries

As shown in Table 95, the instances of phages in the surface water in the intermediate period nurseries were generally few and only at seven investigation points. At of 21 points were 1-2/ml of phages detected. However, in the last period nurseries phages were detected in about one half of the investigation points, and the maximum volume was 13 ml. There were more phages in 1959 than in the corresponding period in 1960.

c) Phages in Paddy Fields

The results of phage investigation in the surface

water of the 41 patches of paddy fields where the seedlings under investigation had been transplanted are shown in Table 96. There was a considerable volume of phage in the intermediate tillering period and the peak tillering period. In some cases it was more than 10^4 /ml before the peak tillering period.

Table 97 shows the results of the quantitative phage test on 2 July 1959, the date of first occurrence, in paddy fields Nos 1, 2, 3, 4, 26, 27, 28, 34, 35, and 39. When these results are compared with the phage volumes on or about 20 June when occurrence was not observable, the great increase in the phage volume in 10 to 12 days can be recognized. According to these results, when $n \times 10^2$ /ml of phages are detected in the surface water of a paddy field, occurrence takes place within less than 10 days, and the phage volume is increased to 10^4 /ml.

Table 98

Relationship Between Nursery Phage Volume and the Phage Volume in the Surface Water During the Intermediate Tillering Period (1959)

Total Phages in Nurseries	Phage in the Surface Water of Paddy Fields 19-21 June /ml							Total
	0	10	50	100	500	1,000	1,000	
30					1		2	3
15					3			3
10				1		1		2
5	1	4	7	1	2			15
0	4	6	5	2	2			19
Total	5	10	12	4	8	1	2	42

Table 99

Relationship Between Nursery Phage Volume and the
Phage Volume in the Surface Water During
the Peak Tillering Period (1959)

Total Phages in Nurseries	Phage in the Surface Water of Paddy Fields, 13-17 July /ml							Total
	0	100	500	1,000	5,000	10 ⁴	10 ⁴	
30							3	3
15							3	3
10				1	1			2
5	0	1	3	3	5	2	1	15
0	0	2	5	2	6	3	1	19
Total	0	3	8	6	12	5	8	42

Table 100

Phage Volume During the Intermediate and
Peak Tillering Periods (1959)

Phage Volume 19-21 June	Phage Volume in the Surface Water in Paddy Fields, 13-17 July /ml							Total
	0	100	500	1,000	5,000	10 ⁴	10 ⁴	
2,000							1	1
1,000					1			1
500						3	5	8
100				1		2	1	4
50			3	3	6	1		13
10			3	3	4			10
0		3	2					5
Total		3	8	7	11	6	7	42

Table 101

Relationship Between Nursery Phage Volume
and Occurrence (1959)

Degree of Occurrence	Total Phage in Nurseries					Total
	0	5	10	15	30	
Extreme	1			1	2	4
Much	4	6	1	2	1	14
Medium	6	9	1			16
Small	6					6
Slight	2					2
Total	19	15	2	3	3	42

Table 102

Relationship Between Phage Volume in the
Intermediate Tillering Period and
Occurrence (1959)

Degree of Occurrence	Phage Volume in the Surface Water of Paddy Fields, 19-21 June /ml						Total
	0	10	50	100	500	1,000	
Extreme					2	2	4
Much		2	7	2	2	1	14
Medium	2	5	5	2	2		16
Small	1	3	2				6
Slight	2						2
Total	5	10	14	4	6	3	42

Table 103

Relationship Between Phage Volume in the Peak
Tillering Period and Occurrence (1959)

Degree of Occurrence	Phage Volume in the Surface Water of Paddy Fields, 13-17 July /ml						Total
	100	500	1,000	5,000	10 ⁴	10 ⁴	
Extreme						4	4
Much		1	1	6	2	4	14
Medium	1	5	4	4	2		16
Small	1	1	1	3			6
Slight	1	1					2
Total	3	8	6	13	4	8	42

The relationships of the phage volume in each period of investigation and the degree of occurrence are shown in Tables 98-103. As can be seen from these tables, when many phages are detected in nurseries, many are detected in paddy fields after transplanting, and the degree of occurrence is also high.

A similar trend was observed in the relationship between the phage volume in the surface water of paddy fields and occurrence by Tagami, *et al*^{69,70,120}. In this experiment, in paddy fields where phages in the surface water in the intermediate tillering period were more than 100/ml, the degree of occurrence was more than "medium." When phages were less than 50/ml, the degree of occurrence was about "small".

2. 1960 Test

1) Test Method

Seventeen nurseries in Naoetsu and Takada Municipalities of Niigata Prefecture were arbitrarily selected, and phages and occurrence in 22 patches of paddy fields with seedlings from these nurseries were investigated according to the following method:

a) Quantitative Phage Test: Twice in nurseries, on 1-3 May, and 19-21 May; twice in paddy fields, on 15-17

June and 1-5 July. Each time, 1 ml of surface water was used for the quantitative phage test.

b) Investigation of Occurrence in Paddy Fields: From 24 to 27 September the degree of occurrence was measured in each patch according to the method of standards for preliminary diagnosis as described in the preceding section.

2) Test Results

Tables 104 and 105 show the phages in the surface water of nurseries in 1960, phages in paddy fields where the seedlings were transplanted, and the degree of occurrence.

3) Results and Observations

a) General Occurrence in the Investigated Area

While 1959 was a year of frequent occurrence, in 1960 occurrence was not observable until 23 July, two months after transplanting to paddy fields (peak tillering period of young ear formation period), and the progress of the disease was slow, so the degree of disease was slight.

b) Phages in Nurseries

As shown in Table 104, instances of phages in the intermediate period of nurseries was small, as in 1960. Although phages were detected at many points in the last period of nurseries compared with 1959, the maximum of phages detected was only 7/ml.

c) Phages in Paddy Fields

A considerable phage volume was detected in the surface water of paddy fields as shown in Table 105. At five points the phage volume was 10^3 per 1 ml. However in general the phage volume was less than that of 1959. This indicated that there is a difference in the volume of phages detected from year to year.

Table 104

Phage Volume in the Surface Water
of Nurseries (1960)

Nursery Number	1-3 May	19-21 May	Total Phage
1	0	0	0
2	0	4	4
3	0	0	0
4	0	1	1
5	0	0	0
6	0	0	0
7	0	0	0
8	0	7	7
9	0	1	1
10	0	2	2
11	1	0	1
12	0	0	0
13	1	1	2
14	1	0	1
15	0	0	0
16	1	0	1

Table
Relationship Between Phage Volume in Nurseries
and in Paddy Fields (1960)

Nursery Number	Total Phages Measured in Field Nurseries	Paddy Field Number	Variety	Phage Volume in Paddy Fields /ml 5-17 June	1-5 July	Degree of Occurrence in Paddy Fields	Date of Dis- covery of First Occurrence
1	0	1	Echiel	16	365	Small	5 August
2	4	2	Nihonkai	7	275	Small	2 September
3	0	3 4	Echiel Morin No. 29	9 8	520 35	Small Slight	2 September " "
4	1	5	Echiel	32	325	Small	25 July
5	0	6 7	Echiel "	30 0	70 685	Slight Small	2 September 5 August
6	0	8 9	Sanin No. 52 Kinnanpu	1 11	770 365	Small "	5 August " "
7	0	10 11 12	Kinnanpu Sanin No. 52 Nihonkai	8 23 21	175 235 460	Slight " Small	5 August " " " "

8	7	13	Kinnanpu	35	3,260	Small	20 July
9	1	14	Kinnanpu	28	1,445	Small	25 July
10	2	15	Bohiei	32	850	Small	5 August
11	1	16	Bohiei	10	35	Slight	2 September
12	0	17	Korin No. 29	34	255	Slight	5 August
13	2	18 19	Bohiei Kinnanpu	22 137	155 2,480	Slight Medium	2 September 25 July
14	1	20	Nihonkai	0	405	Small	2 August
15	0	21	Bohiei	50	1,345	Small	25 July
16	1	22	Kinnanpu	42	2,935	Medium	25 July

Table 106

Relationship Between the Phage Volume in Nurseries
and the Phage Volume in the Surface Water
During the Intermediate Tillering
Period (1960)

Phages in Nurseries	Phages in the Surface Water of Paddy Fields 15-17 June /ml					Total
	0	5	10	50	50	
10		1				1
5			1	2	1	4
1	1		1	3		5
0	1	1	3	7		12
Total	2	2	5	12	1	22

Table 107

Relationship Between the Phage Volume in Nurseries
and the Phage Volume in the Surface Water of
Paddy Fields in the Peak Tillering
Period (1960)

Phages in Nurseries	Phages in the Surface Water of Paddy Fields, 1-5 July / ml					Total
	50	100	500	1,000	5,000	
10					1	1
5			2	1	1	4
1	1		2		2	5
0	1	1	7	2	1	12
Total	2	1	11	3	5	22

Table 108

Phage Volume in the Surface Water of Paddy
Fields in the Intermediate and Peak
Tillering Periods (1960)

Phages in Nurseries	Phage Volume in the Surface Water in Paddy Fields, 1-5 July / ml					Total
	50	100	500	1,000	5,000	
50					1	1
50		2	7	1	3	12
10	2		2	1		5
5				1		1
0			1	1		2
Total	2	2	2	10	4	22

d) Relationship Between Phages in Nurseries and
Phages in Paddy Fields

The mutual relationship between phages in the surface water of nurseries and paddy fields detected in each period of investigation is shown in Tables 106-108.

The relationships of the volumes of phages in nurseries, on the surface of paddy fields in the intermediate and peak tillering periods, and the degree of occurrence are shown in Tables 109-111.

Table 109

Relationship Between the Phage Volume in
Nurseries and Occurrence (1960)

Degree of Occurrence	Phage in Nurseries				Total
	0	1	5	10	
Extreme					0
Much					0
Medium		1	1		2
Small	7	3	2	1	13
Slight	5	1	1		7
Total	12	5	4	1	22

Table 110

Relationship Between the Phage Volume in
the Intermediate Tillering Period
and Occurrence (1960)

Degree of Occurrence	Phage in the Surface Water in Paddy Fields, 15-17 June /ml					Total
	0	5	10	50	50	
Extreme						0
Much						0
Medium				1	1	2
Small	2	1	3	7		13
Slight			3	4		7
Total	2	1	6	12	1	22

Table 111

Relationship Between the Phage Volume in
the Peak Tillering Period and
Occurrence (1960)

Degree of Occurrence	Phage in the Surface Water of Paddy Fields, 1-5 July /ml					Total
	50	100	500	1,000	5,000	
Extreme						0
Much						0
Medium					2	2
Small			7	3	3	13
Slight	2	1	4			7
Total	2	1	11	3	5	22

As is clear from the foregoing tables, the relationship between the phage volume detected in nurseries and the phage volume in paddy fields was not certain, because the former was only in a small amount. The relationship between the phage volume in each period of investigation and occurrence in 1960 was not clear-cut, either, as in 1959.

However, it is noteworthy that when the phage volume detected in nurseries was not great, the disease occurrence in paddy fields was not great either.

3. Summary

The results of tests on the relationship between the phage volume in the surface water at 10 points in nurseries and paddy fields in Takada and Naoetsu Municipality, Niigata Prefecture, and occurrence, in 1959 (heavy occurrence year) and 1960 (light occurrence years) are summarized in the following:

1) The phage volume detected in the intermediate period nurseries (mid-May) was generally small. It was less than three per one ml in a year of heavy occurrence. It tended to increase somewhat in the latter period nurseries (late May). However, even in a year of heavy occurrence, the highest was 26 per 1 ml.

2) Phages seemed to increase after transplanting to paddy fields. It was 10^3 in the intermediate tillering period in 1959, and over 10^4 in the peak tillering period. In such paddy fields, occurrence was observable, and damage caused thereafter was also great.

3) When many phages are detected in nurseries even after transplanting to paddy fields, the phage volume is much more than in other cases, and the degree of occurrence is also great.

4) In the case of paddy fields, where the phage volume in the surface water in the intermediate tillering period is over 100 per 1 ml, the degree of occurrence is about "medium," and if it is less than 50 per 1 ml, the degree is about "small."

5) When the phage volume detected in nurseries is small, in the same year, the occurrence after transplanting to paddy fields is also small.

Section 3. Relationship Between the Phage Volume in Irrigation Water and Occurrence 165

In the preceding section, phages in the surface water of individual nurseries or of paddy fields was quantitatively tested, and the relationship between the phage

volume and occurrence was investigated. In this test, the points for phage measurements were set up in places other than in the surface water of paddy fields; in such places as irrigation channels or reservoirs in constantly infected locations. The quantitative fluctuation of phages at such places, and the occurrence process in the vicinity were tested. The test was conducted at Naoetsu Municipality, Niigata Prefecture (the constantly infected areas of Aono-tomaji, and Koshiyanagi, 800 x 900 m, about 70 ha.).

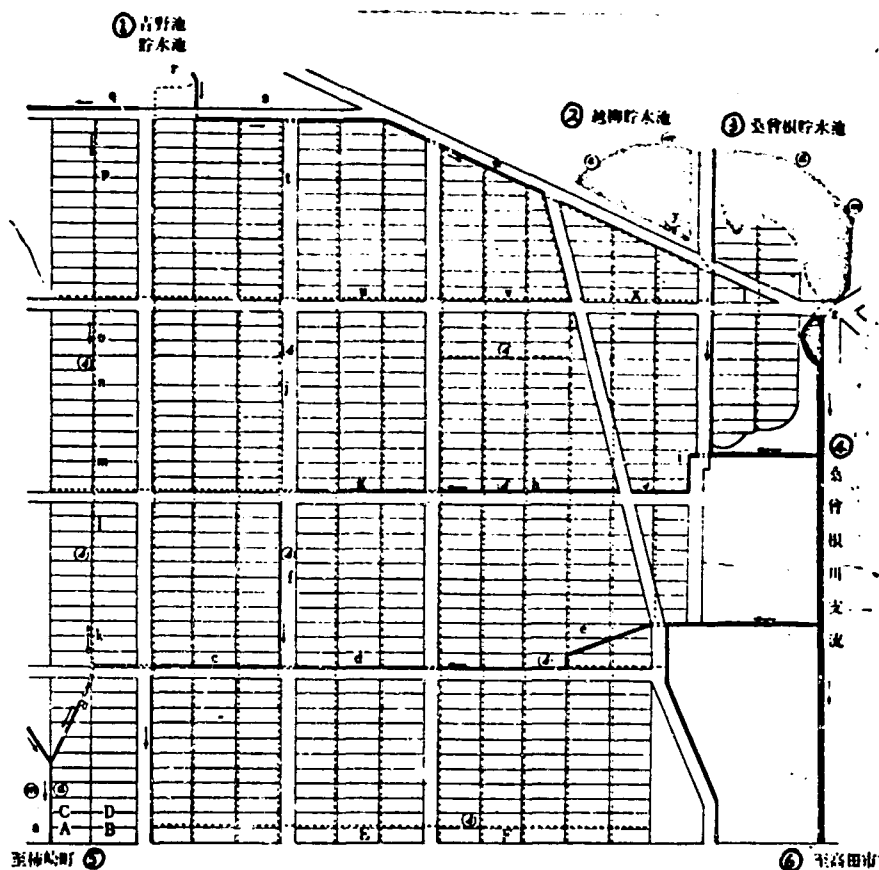


Fig. 24 Reservoir and Irrigation Channel System in the Aono-tomaji and Koshiyanagi Area of Naoetsu Municipality (1959)

[Legend]: 1) Aono Reservoir; 2) Koshiyanagi Reservoir; 3) Kuwasone Reservoir; 4) Tributary of Kuwasone River; 5) To Kakizaki-cho; 6) To Takada Municipality; a-z) Points of water collection; (S) *Leersia oryzoides* (Linn.) Sw.; (Z) *Zizania latifolia* Turcz.; (a))

Leersia japonica Makino; A-F) Points of collection of surface water from paddy field.

1. 1959 Test

1) Test Method

As shown in Figure 24, an area with a comparatively clear irrigation channel system was selected. Phages from several points in irrigation channels and reservoirs included in this area, were quantitatively tested in each growth period, from the first period in paddy fields to the ear formation period, as shown in Table 112. Concurrently, the occurrence process in the area was also investigated.

2) Test Results and Observations

Test results are shown in Tables 112-114.

As shown in Figure 24, irrigation water in this area is supplied by the Kuwasone Reservoir, in the southeastern part, Koshiyanagi Reservoir, and Aono Reservoir, and the source and channels of irrigation water are comparatively clear. Phage investigation was carried out at a-z points, as shown in Figure 24. At the same time, the fluctuation of disease occurrence in the area in general was observed. The results are shown in Tables 112 and 113. At each point, the increase of phages detected with the elapse of time was noted. In this test, 45 phages per 1 ml were detected at point "a" on general drainage in the investigation area immediately after transplanting. However phages in the water source and in the irrigation channel for the same period were less than this. Thus there seems to be a trend for phages to increase as they reach the lower course.

In these tests, as phage indicators, Shinjo strain (lyso-type A) and Benikonaya strain (lyso-type B) were used. Until 6 June (intermediate tillering period and third investigation) more phages were detected by lyso-type A bacteria, while up to 24 June (peak tillering period and fourth investigation) the volume of phages detected by the two types of bacteria were nearly the same. At some places more phages were detected by lyso-type B. However, as shown in Table 112, it was estimated that as a whole, the life cycle of phages could be grasped by lyso-type A bacteria alone. This kind of thing should be decided by the phages distributed in the concerned area. However, in this area much of the lyso-type A bacteria was isolated, and there was much OP₁ with affinity to lyso-type A bacteria, as was determined by the test which will be described in

Table 112

Volume of Phage in Reservoirs and Irrigation Channels in the
Aono-tomoji Area of Naoetsu City, and Occurrence Process
(See Figure 24 for Points of Investigation)

Indicator Investigation		25 May	29 May	6 June	24 June	15 July	24 July	4 Aug.	Remarks
Strain	Points								
Shinjo Strain (type A) Irrigation Channel and Reservoir	a	45	42	33	66	1,780	4,180	6,920	General drainage
	b		23	36	208	2,350	5,160	4,450	Growth of
	c		78	80	276	1,260	3,900	4,160	Zizania lati-
	d			53	40	428	4,500	3,440	folia Turcz,
									Leersia Japo-
	e			25	80	418	2,090	290	nica Makino
	f		133	44	199	1,350	5,040	4,255	Growth of Leer-
	g			48	109	832	5,080	1,575	sia oryzoides
	h		78		95	640	3,840	865	(Linn.) Sw.
	i		161	16	114	220	5,120	2,655	
	j		65	66	274	2,200	885	5,320	
	k		69		106	1,850	5,550	4,620	
	l		56					1,765	
	m		33		142	1,610	1,357		
	n			100	199			775	
	o				161	2,120	1,275		
	p		12	122	248	900	2,080	2,040	

q	8	44	88	15	15
r	0		0	0	0
s					25
t	24				

Aono Reservoir

u	38				1,215
v					2,520
w	3				40

Irrigation
Channel
and
Reservoir

x	15	9	0	10	87
y	1				

Koshiyanagi
Reservoir

z	3	81	52	845	340
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Tributary of
Kuwasone River

Shirjo
Strain
(type A)

1 222 1

A	122	2,100	3,280	Low, wet field
B	10	7,040		Occurrence on
C		9,000		15 July "
D		10,000		"
E			70	Non-occurrence
F			8,500	field

Surface
Water of
Paddy
Field

Non-occurrence
field
Infected field
24 July

G	142			Inundated field
H	8			"
I	19			Non-inundated
J			2,040	field
				Inundated Field

z	1	0	30	86	550	170
Benikonaya Field Strain (type B)	Paddy Surface Water	A	85	2,800	3,100	
		B		5,000		
		C	2	6,400		
		D		10,000		
		E			330	
		F			2,410	
G	H	I	J	166		
				26		
				8		
					250	

Mean Temperature (°C) 16.1 18.2 21.5 21.9 24.8 26.0 26.0

Occurrence No occur- Same as Same as * ** Full Remark-
rence left left left occurrence able
disease
progress

* At point j, the first occurrence of Leersia oryzoides (Linn.) Sw. was discovered. Occurrences at other places were estimated.
** Occurrence of Leersia oryzoides (Linn.) Sw. was observed in paddy fields, A,B,C, and D, and at points d, i, j, and m.

Table 113

Comparison of Phage Volumes in the Water and
in the Lower Course of the Irrigation
Channel (excerpts from Table 112)

Point		Reservoir*					→ Drainage ^o		
Date of Phage Measurement	z*	i	h	g	f	c	b	a ^o	
29 May	3	161	78	-	138	78	23	42	
6 June	8	16	-	48	44	80	36	33	
24 June	81	114	95	109	199	276	208	66	
15 July	52	220	640	832	1,350	1,260	2,350	1,780	
24 July	845	5,120	3,840	5,080	5,040	3,900	5,160	4,180	
4 August	340	2,655	865	1,575	4,255	4,160	4,450	6,920	

Table 114

Phage Volume at Investigation Points at the Time
of the First Occurrence of Leersia oryzoides
(Linn.) Sw. and Rice Plants

Date	Point	Bacteria Detected		Point	Bacteria Detected	
		Type A	Type B		Type A	Type B
	j	274	263	d	428	275
<u>Leersia oryzoides</u>	(b)	(206)	(341)	i	220	156
(Linn.) Sw.				m	1,610	584
Rice				a	1,780	750
				j	2,200	556
				d	428	275
				(b)	(2,350)	(830)

Note: Point B (See Figure 24) was the confluence of all irrigation water channels. This was used as a comparison.

Section 5. Therefore, the following observation was made about the amount of phage detected by lyso-type A bacteria.

As has been stated, the number of phages in the reservoir, the source of irrigation water, was less than in the same period (for instances, points r, y, z in Table 112). More phages were found in the general drainage than in the water source or in irrigation channels. This seems to be due to the multiplication of bacteria overwintering in Leersia oryzoides (Linn.) Sw. or Zizania latifolia Turcz that naturally grow in irrigation channels and field ridges in the area, or to the increased phages, because of bacterial multiplication in rice of infected paddy fields, which are dumped into irrigation water channels. This then results in a general increase of phages in the lower parts of irrigation channels. Thus, in an area where the water system is comparatively simple and clear, more phages are found in the lower reaches of water channels than in the upper reaches which serve as the source of irrigation water. And the number of phages at each point is dependent on whether Leersia oryzoides (Linn.) Sw. already infected or about to be infected exists in the vicinity, on the bacterial multiplication in nearby paddy fields, on whether or not disease has occurred in rice plants, and on their distances and locations. These estimations may be endorsed by points f, i, and j (colonies of Leersia oryzoides (Linn.) Sw.) which were surveyed on 29 May and 6 June, as shown in Table 112.

As far as the relation between fluctuations in numbers of phages and occurrence, it varies with points of investigation. As shown in Table 114, in the irrigation channels in paddy fields, it is 40-300/ml in the latter part of June, and at this time the first occurrence in Leersia oryzoides (Linn.) Sw. is observable. Then, on 15 July 1,000-2,000/ml of phage is detected, and the first occurrence in rice is discovered. At the same time, more than 7,000-10⁴/ml of phage is detected in the surface water of paddy fields. The amount detected at the time of occurrence in paddy fields is almost equal to that in the test described in the preceding section. In other words, the number of phages at the time of occurrence is 1,000-2,000/ml in irrigation channels, while it is 10⁴/ml in the surface water of paddy fields. Although there was no survey made in the early part of July, the first occurrence in Leersia oryzoides (Linn.) Sw. was estimated to be approximately 10 June, and that in rice, the early part of July. Therefore, it was estimated that the number of phages in the period of first occurrence in Leersia oryzoides (Linn.) Sw. in irrigation channels would be 30-80/ml. Next, after

the discovery of occurrence in rice plants, the number of phages detected increased with the increase of infected paddy fields and with the progress of occurrence. With the stagnation or cessation of occurrence, the number of phages also decreased. Thus a close relationship between the number of phages and occurrence was recognized.

2. 1960 Test

1) Test Method

At the same places and according to the same method as 1959, the test was conducted. In this test, only the Shinjo strain (type A) was used as the phage indicator. Measurement points were identical with those ones of 1959, as shown in Figure 25. In 1960, the number of phages in the surface water of paddy fields at 16 points in this area, with special reference to the number of phages upto the time of occurrence was investigated.

2) Test Results and Observations

The amounts of phage, occurrence, at each point of investigation are shown in Figure 25, and the climatic conditions during the period of investigation are shown in Tables 115 and 116.

As shown in Table 115, there was considerable difference in the number of phages detected depending on where samples were collected. Phages gradually increased beginning with the first period in paddy fields, and on 27 July (before young ear formation period), 1,000-2,000/ml of phage was detected in irrigation water channels, and occurrence was observed in four out of 16 patches. The number of phages in the surface water of infected paddy fields was 10^4 /ml as in the preceding year. The relationship between the number of phages and occurrence was similar to the preceding year. However, while the number of phages on 7 July, when the first occurrence was discovered in Leersia oryzoides (Linn.) Sw., was 50-100/ml in irrigation water channels, that of the first occurrence in the preceding year was 40-300/ml.

The number of phages varies greatly at points r, s, t, u, v, w, and y in Figure 25. All of these places are located in the upper reaches connected to the reservoir. The number of phages detected increases as it descends toward the lower reaches. A similar trend as in the investigation of the preceding year was observed.

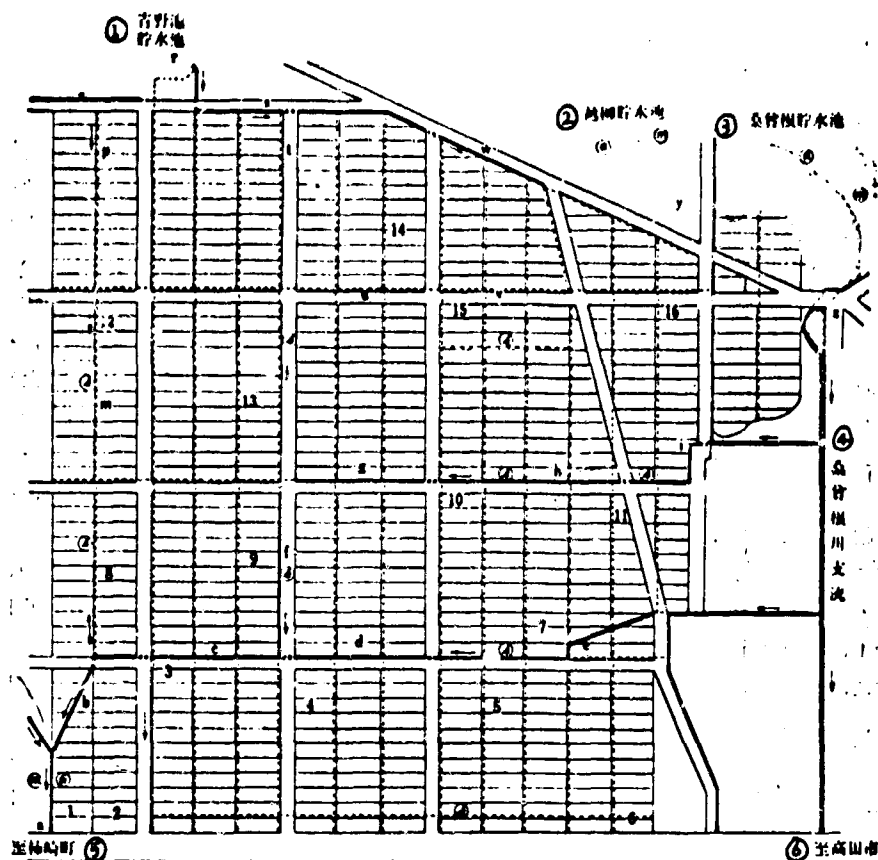


Fig. 25 Reservoir and Irrigation Channel System in the Aono-jumonji-Koshiyanagi Area, Naoetsu Municipality (1960)

[Legend]: 1) Aono Reservoir; 2) Koshiyanagi Reservoir; 3) Kuwasone Reservoir; 4) Tributary of Kuwasone River; 5) To Kakizaki; 6) To Takada Municipality; (S) *Leersia oryzoides* (Linn.) Sw.; (m) *Zizania latifolia* Turoz; (a) *Leersia japonica* Makino; a-z) Points of water collection 1-16 paddy field numbers for the investigation of occurrence.

Table 115

Phage Fluctuation in the Reservoirs and Irrigation
Channels in the Aono-Jumonji Area of
Naoetsu Municipality (1960)

①地点番号		5月22日	5月29日	6月7日	6月16日	6月28日	7月7日	7月14日	7月27日	8月6日	8月15日
④灌 溉 水 路・貯 水 池	a b c d	1 0 0	5 4 10	12 15 13 12	29 39 23 44	46 47 60 31	68 75 97	408 480 363 191	1,060 4,840 1,820	540 1,777 500	2,320 4,606 2,760
	e f g h	0 0 2	0 0 5 1	6 3 8 5	21 23 79 97	121 16 2 39	90 109 80	390 70 528 157	1,050 1,620 2,130 2,270	440 260 322 460	1,134 2,255 3,980 2,280
	i j k l		0 0 6	11 2 14	99 11 41	44 27 58	75 93 79	28 333 187	90 460 1,090	161 710 1,610	1,485 1,110 4,600
	m n o p		1 3	12 1	43 18	69 22	83 54	116 45	2,100 1,600	820 260	1,700 670
	q r s t	0	0 1	0 2 0	2 2 1	1 1 2	3 1 10	4 1 16	0 0 29	0 0 110	1 2 178
	u v w x		3	8	15 2	29 4	27 4	153 2	198 10	65 1	216 0
	y z	0	0	1 3	2 2	2 4	0 15	1 21	2 62	8 122	1,103
⑤本 田 面 水	1 農林29号 ⑤	0	5	21	37	82	439	5,962	N	2,420	
	2 "	2	6	17	12	79	155	2,197	9,200	2,517	
	3 山陰52号 ⑦	2	5	22	35	58	290	2,313	N	3,722	
	4 日本海 ⑧	0	3	30	14	90	384	10,840	N	5,290	
	5 山陰52号	1	6	27	31	0	48	76	1,266	2,230	
	6 越 栄 ⑨	1	4	13	10	75	155	2,870	N	6,410	
	7 越路早生 ⑩	0	5	17	19	80	361	14,980	N	9,285	
	8 日本海	2	6	22	33	85	172	12,200	N	11,200	
	9 キンマサ リ ⑪	3	7	13	34	60	169	7,300	N	7,710	
	10 短銀坊主 ⑫	4	26	47	—	118	950	12,000	N	13,910	
	11 金 南 風 ⑬	1	0	17	13	56	181	3,200	N	4,800	
	12 米 山 ⑭	2	7	10	3	17	70	550	760	240	
	13 北陸52号 ⑮	1	0	1	12	25	49	428	1,030	120	
	14 農林29号	3	1	3	17	33	64	303	920	130	
	15 山陰52号	0	2	5	21	25	79	49	2,300	70	
	16 北陸52号	2	3	7	24	34	101	72	3,800	290	

⑤ 注: Nは計算不能なほど多いことを示し, ~は発病中の水田の田面水ファージ量を示す。

Legend: 1) Survey point number; 2) Month;
3) Day; 4) Irrigation channels and reser-
voirs; 5) Surface water of paddy fields; 6)
Norin No. 29; 7) Sanin No. 52; 8) Nihonkai;
9) Echiei; 10) Koshiji early maturing; 11)
Ginmasari; 12) Short ginboze; 13) Kinnanpu;
14) Yoneyama; 15) Hokuriku No. 52; 16) Note:
N shows that phages are too numerous to be
counted. ~ shows the number of phages in
the surface water of infected paddy fields.

Table 116

Occurrence and Climatic Conditions in the Area Under Investigation

Date	May		June		July		August			
	22	29	7	16	28	7	14	27	6	15
<u>Weather</u>										
<u>Weather</u>										
Previous Day	○	●	●	○	●	●	●	○	○	●
Current Day	○	●	●	●	●	●	●	●	○	○
<u>Temperature</u>										
Highest	22.6	20.7	18.7	23.3	27.7	23.0	29.4	31.4	32.5	27.5
Lowest	8.3	16.5	15.5	17.3	19.0	18.4	21.8	24.5	24.4	16.5
Mean	15.9	16.7	15.9	19.6	23.4	20.3	25.1	28.4	28.5	22.8
<u>Precipitation</u>										
Continuous light rain in May	Continuous rain in the first half of July									
	3-4 July 30mm									
	9-10 July 30mm									
10 May 25mm	23 July-10 August, fine weather with high temperature and dryness									
29 May 20mm										
<u>Occurrence</u>										
Occurrence was observed in <u>Leersia oryzoides</u> (Linn.) Sw. on 28 June	Occurrence in <u>Leersia oryzoides</u> (Linn.) Sw. on 7 July									
	Discovery of first occurrence in rice on 23 July									
	Occurrence at several places on 6 August (Full occurrence period)									

What attracted attention in this investigation were, the phage increase period was very slow as compared with the corresponding period of the preceding year, the rate of increase was very slow, and the number of phages detected was less than in the corresponding period of the preceding year. These differences seemed to be yearly fluctuations. The factor behind these fluctuations seemed to be the period of the beginning of multiplication of the pathogen of rice bacterial leaf blight and its multiplication process. Therefore, it was estimated that indirect forecast and estimation of bacterial multiplication and occurrence will be possible by the periodic quantitative test for the number of phages in the irrigation water channel, as conducted in the present test.

3. Summary

For two years, from 1959 to 1960, phage in irrigation channels and reservoirs in the constantly infected Aono-Jumonji and Koshiyanagi area of Naoetsu Municipality, Niigata Prefecture was measured, and the occurrence in the area was investigated. From these the following was clarified.

1) Phages in irrigation channels increase from the early period of paddy fields. However, when the increase reaches 1,000-2,000/ml, occurrence in nearby paddy fields is observable.

2) Phage in irrigation channels increases as it proceeds from the water source toward the lower reaches.

3) The number of phages in the surface water of paddy fields at the time of occurrence is over 10^4 /ml.

4) The number of phages in the irrigation channels at the time of first occurrence in Leersia oryzoides (Linn.) Sw. varies with years, but it is generally about 100 per 1 ml.

5) The fluctuation in the number of phages after occurrence corresponds to the increase in infected paddy fields, the progress of the disease, or to stagnation. Thus there seems to be a close relationship between phage fluctuation in irrigation channels and occurrence.

6) After the observation of the first occurrence in rice (especially in the second period of occurrence in the latter half of rice growth), the number of phages does not increase or decrease with the progress or cessation of occurrence.

7) Since there are differences in phage fluctuation detected at the same points of yearly measurements, it seems possible to estimate the time and degree of occurrence.

Section 4. Relationship Between the Number of Phages in Rivers and Channels, and Occurrence in the Same Area¹⁶⁵

In Sections 2 and 3, the establishment of points for the quantitative phage test in nurseries and paddy fields was described, and in the irrigation channels in particular areas, the quantitative phage change in irrigation water, and their relation to the occurrence of rice bacterial leaf blight in paddy fields nearby. In the present test, phages in rivers and main water channels flowing through a larger area of about 2 km in radius with the Hokuriku District Agricultural Experimental Station at the center, and its relationship to the occurrence of rice bacterial leaf blight in the area was examined. The present test lasted for four years from 1958 to 1961.

1. 1958 Test

1) Test Method

An area 2 km in radius with the Hokuriku District Agricultural Experimental Station as the center was selected as the object of survey. Phages were measured in the Arakawa River where water channels and drainage converge, in the Daido water channel which serves as the main irrigation channel, and in the Koyasu channel which is a branch of the Chuko channel, beginning in April. The quantitative phage test followed the method described in Section 1. In 1958, Shinjo strain (type A) was used as the indicator strain. The time of collection of sample irrigation water was from 10:00 a.m. to 3:00 p.m.. Next, the occurrence of disease in Leersia oryzoides (Linn.) Sw. and in rice in the area under survey was observed and recorded with a field survey at about 10 day intervals.

2) Test Results and Observations

Test results are shown in Table 117.

For the year 1958, preliminary detection of phage in the irrigation channels and reservoirs in the fields of the Hokuriku District Agricultural Experimental Station was

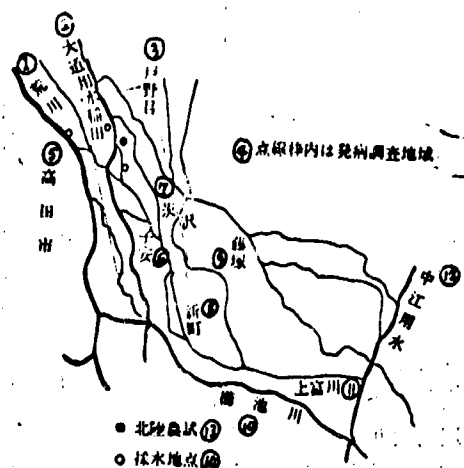


Fig. 26 Water System in the Vicinity of the Hokuriku District Agricultural Experimental Station

[Legend]: 1) Arakawa; 2) Rice paddy fields in Daido channel; 3) Tonome; 4) Area demarcated by dotted line is the area under investigation; 5) Takada Municipality; 6) Koyasu; 7) Ibarazawa; 8) Shinmachi; 9) Fujizuka; 10) Kushichi River; 11) Kamito-mikawa; 12) Chuko water channel; 13) Hoku-riku District Agricultural Experimental Station; 14) Water Collection points.

attempted on several occasions beginning in the latter part of March. The first detection was, as shown in the note to Table 117, on 1 May in the water of the reservoir (where Leersia japonica was growing) within the station. Thereafter a small amount of phage was detected in Arakawa and other channels. Then the amount increased and on 27 June the first occurrence was observed in Leersia oryzoides (Linn.) Sw. growing along the irrigation channels in the Station. At this time, the amount of phage was 17 per 1 ml in the Arakawa River, 46 at the Daido channel, and 43 at the Koyasu water channel. After July, more was detected, and the lowest temperature at this time in the Takada area was above 20°C. By this time bacterial multiplication seemed to become active in rice and other intermediate host plants along field ridges. In mid-July, 200-300 phages were detected in the Arakawa River, Daido, and Koyasu channels. Then at several points in the investigated area the first occurrence in rice was observed. The number of

Table 117
Relationship Between Phage Fluctuation in Rivers
and Channels and Occurrence (1958)

Date	Arakawa	Daido	Koyasu	Temperature of Paddy Field (°C)	Occurrence
3 April	0	0	0		First occurrence in <u>Leersia ory-</u> <u>zoides</u> (Linn.) Sw. on 27 June.
1 May	0	0	0	18.5	First occurrence in rice was dis- covered at several places on 16-19 July
8 "	0	0	6	15.5	
28 "	3	1	6	18.8	
9 June	2	4	10	21.2	Typhoon No 11 passed on 22-23 July with heavy rain afterwards. Disease showed marked progress.
20 "	17	46	43	23.2	
1 July	19	47	45	24.0	Disease among late maturing vari- eties showed marked progress due to the impact of typhoons Nos 21 and 22 (17, and 26 September).
9 "	38	36	160	29.5	
17 "	275	395	260	23.5	
28 "	343	2,100	3,560	26.5	
6 August	1,600	1,740	4,520	24.0	
14 "	76,000	92,000	20,000	24.2	
22 "	6,100	12,000	16,100	25.3	
3 September	1,300	3,300	159,000		
17 "	4,300	3,200	3,500		
30 "	600	1,200	400		

1 October	3,200	14,500	1,300
4 "	0	33	120
11 "	4	3	87
18 "	3	4	47
25 "	2	3	7

2 December	1	1	19
9 "	1	7	20
16 "	1	1	1
23 "	0	1	1

Note: At the reservoir in the Hokuriku District Agricultural Experimental Station, when phage was measured as a comparison, two phages per 1 ml were detected on 1 May. For the phage indicator, Shinjo strain (type A) was used.

phages rapidly increased after mid-July with the increase of diseased area in rice, the progress of occurrence, and immersion caused by Typhoon No 11 on 25 July. Therefore much phage was detected from mid-August to early September. Especially on 14 August when it was flooded by rain, the number of phages rapidly increased, and a maximum of $5-15 \times 10^4$ per 1 ml was recorded. However, after mid-September, the number of phages showed a gradual decrease and declined greatly in November. This was estimated to have been caused by the cessation of bacterial multiplication in host plants with the fall in temperature because of the harvest of rice plants.

As far as the number of phages detected at each investigation point was concerned, no great difference among points was noted with the exceptions of a trend toward more phages in the Daido and Koyasu channels on 20 June.

As shown in the foregoing, the relationship between phage fluctuation in rivers and channels, and the occurrence and occurrence process in paddy fields in the area were related to the climatic conditions of the time, and seemed to be show a parallel relationship.

2. 1959 Test

1) Test Method

Place and method were generally the same as those in 1958. However in this test, as phage indicators, Shinjo strain (type A), and Benikonaya strain (type B) were used. And, at the time of water collection, water temperature at each point was measured.

2) Test Results and Observations

The results obtained from the foregoing method are shown in Table 118.

On 23 April 1959 (the early period in nurseries), phage was detected in the Koyasu channel. This was two weeks earlier than in 1958. Detection was until June, and in comparison with that of 1958, there were more phages detected at each point of investigation. On 21 June, the first occurrence in Leersia oryzoides (Linn.) Sw appeared in the Koyasu channel. This was one week earlier than in 1958. On 2 July, the first occurrence in rice was observed in the fields of the Agricultural Station, or two weeks earlier than in 1958. The number of phages at these times was over 10-21 per 1 ml at the time of the occurrence in

Leersia oryzoides (Linn.) Sw., and over 93-95 at the time of the first occurrence in rice. These figures were very close to the 1958 figures.

As shown in the occurrence column, infected fields showed zurikomi symptoms in late June in the upper reaches (Tomikawa area) in the area under investigation. The same symptoms appeared at several places in the upper reaches. It seemed the impact was shown in early phage detection and in the number of phages detected during May. Generally speaking, occurrence in the whole area was earlier than in 1958. After heavy rain on 1 and 2 July (114 mm) and on 11 July (160 mm), paddy fields were flooded and this increased the area of occurrence, reaching the highest record in this area. Correspondingly, the number of phages increased with occurrence progress. Thus in late July, about 2,000 phages per 1 ml were detected. However, because of the fine weather, lasting from mid-July to early August, the progress of the disease showed considerable stagnation, and the number of phages detected was less than 10^4 /ml. In early August the number detected showed a temporary decline; a trend toward the summer cessation period as clarified at the time of the investigation of bacterial fluctuation in rice leaves.

There were several typhoons between mid-August and mid-September, but all of them were weak and without rain. Thus, their impact was negligible. After October, the temperature fell and the disease began to end. Thus while there were several places with severe infection in the early period of growth, damage caused by the disease overall was rather small. The number of phages detected in the first half of rice growth was far less than in 1958, and occurrence was correspondingly far less severe. It seems that there is an interrelationship between the number of phages and the process of occurrence.

In this investigation, Type A and B strains were used as phage indicators. As a whole there were more phages detected by Type A, and less with an affinity to Type B bacteria throughout the investigation. In the area under investigation OP₁ and OP₂ were distributed. It was learned from separate tests that the former was predominant over the latter. It seemed sufficient to consider, the relationship of occurrence on the basis of the data detected by Type A bacteria alone.

Table 118
Relationship Between Phage Fluctuation in Rivers
and Channels and Occurrence (1959)

Place and Date of Investigation	Arakawa		Daido Channel		Koyasu Channel		Occurrence
	Type A	Type B	Type A	Type B	Type A	Type B	
23 April	0	0	1	0	6	1	Occurrence was observed on 21 June in Leersia oryzoides (Linn.) Sw. growing in the Koyasu channel. At about the same time severe occurrence with the wilting symptoms was noticed in the upper reaches of the Koyasu channel in the Tomikawa area.
1 May	0	1	5	0	21	0	
11 "	3	0	2	2	4	0	
20 "	0	0	3	0	2	0	
13 June	7	0	8	0	19	0	By early July occurrence began to spread generally. The first occurrence was observed in the paddy fields of the Hokuriku Station, on 20 July.
18 "	10	0	21	0	17	0	
30 "	95	4	93	7	73	0	
13 July	130	5	420	19	148	92	Fine weather continued from mid-July to mid-August.
20 "	415	20	495	170	860	320	
27 "	2,890	45	2,140	95	1,810	120	
16 August	260	10	1,170	20	680	10	
28 "	1,390	20	2,400	10	2,040	50	
5 September	1,150	5	1,570	10	3,710	505	
16 "	30	5	20	10	1,510	885	
28 "	505	6	3,545	34	2,200	546	

10 October	15	0	70	5	160	2	Typhoons Nos 14 and 15 passed on 18 and 27 September, but without impact on the progress of occurrence because of their weakness.
20 "	22	1	134	10	350	235	
5 November	31	2	7	2	324	14	
13 "	6	0	15	0	40	0	
24 "	2	1	23	13	53	7	
4 December	25	0	68	1	22	4	
14 "	0	0	0	0	2	0	
24 "	2	-	2	-	0	-	
11 January	0	0	0	0	0	0	

Note: For Type A bacteria, Shinjo strain, and for Type B bacteria Benikonaya strain were used.

3. 1960 Test

1) Test Method

From the test results so far, the places for investigation were concentrated on the three points of Arakawa, Daido channel, and Koyasu channel. Type A bacteria (H5820) was used for detection. Five patches of paddy fields in the area were selected for the study of occurrence progress, and they were compared with the quantitative phage test. These five patches are shown in Table 120. (For their locations see Figure 26).

2) Test Results and Observations

Test results from the above are as shown in Tables 119 and 120.

In 1960, phage was detected rather late, on 21 May. This was 10-20 days later than in 1958, and about 30 days later than 1959. This was because while the mean temperature in April of 1960 was 10.4°C, that of 1958 was 11.2°C, and it was 12.6°C in 1959. In other words, the mean temperature in April 1960 was lower than in any other year. Especially in the first half of April of this year the highest, lowest and mean temperatures were lower by 3°C. This factor, it was estimated, delayed bacterial multiplication and then phage multiplication from the first to the intermediate nursery period. However, the temperature from late May, after transplanting, to mid-July was normal, and an increase in the number of phages detected was observed. On 10 July occurrence in Leersia oryzoides (Linn.) Sw. was observed in the ridges along the Koyasu channel. On 25 July, as shown in Table 120, occurrence was observed in the rice in three patches of paddy fields under investigation, the paddy fields in the station and its vicinity. The number of phages in this period was 18-167 per 1 ml on 8 July when occurrence in Leersia oryzoides (Linn.) Sw. was observed, and 345-900 on 18 July when the first occurrence in rice was observed. These amounts were roughly equal to those of the preceding two years.

After occurrence in rice was observed, the increase in the area of occurrence, the progress of the disease, or its temporary stagnation corresponded well to the phage fluctuations. Thus it rapidly increased from late July when the first occurrence was observed to early August, reaching a peak. In mid-August and early September the amounts detected showed a temporary decline, followed by an increase again in mid-September, and a rapid decline in October.

Table 119

Phage Fluctuation in Rivers and Channels (1960)

場①所 事項③ 月日⑥	② 荒 川				③ 大 込 用 水				④ 子 安 用 水			
	フアージ 量 1ml 当り⑦	水温 (°C)⑧	⑨ 水位	⑩ 濁度	フアージ 量 1ml 当り	水温 (°C)	水位	濁度	フアージ 量 1ml 当り	水温 (°C)	水位	濁度
4. 9	0	5	平⑪		0	7	平		0	13	減	
19	0	10	増⑫		0	12	平		0	14	増	
29	0	10	増		0	12	平		0	12	増	
5. 9	0	8	平		0	11	減		0	12	増	
21	1	16	増		1	16	減		3	17	増	
30	1	15	増	濁⑬	7	15	増	濁	9	17	増	濁
6. 8	4	23	平	透⑭	7	17	増	半濁	11	19	平	透
18	18	23	平	半濁⑮	29	22	増	濁	24	22	平	透
30	31	21	平	濁	85	12	増	濁	76	23	減	透
7. 8	18	24	減⑯	半濁	165	12	増	濁	90	22	減	透
18	345	24	平	半濁	900	22	平	半濁	170	28	平	半濁
29	23,300	23	減	半濁	6,700	23	増	濁	1,500	25	減	透
8. 8	5,150	30	減	透	4,600	29	増	透	2,600	32	増	濁
18	300	27	減	透	500	27	増	半濁	250	27	増	透
29	1,200	28	減	半濁	1,100	27	減	透	3,000	27	平	透
9. 9	170	24	平	透	550	23	減	濁	620	27	減⑰	透
17	4,790	23	平	半濁	13,200	23	減	濁	210	26	溜水	透
10. 1	50	15	平	半濁	35	16	減	濁	40	17	溜水	透
16	2	—	平	透	16	—	減	透	65	—	溜水	透

The Process of Occurrence The occurrence of disease was observed in Leersia oryzoides (Linn.) Sw. water channels on 10 July.

The occurrence of the disease in rice was discovered on 18 July, much later than usual.

From 22 July to 9 August, unusual high temperatures and dryness continued, and the disease showed a temporary decline.

But after 7 September, because of the continuous rain, the disease showed considerable progress in the late maturing varieties.

[Legend]: 1) Place; 2) Arakawa; 3) Daido Channel; 4) Koyasu Channel; 5) Itea; 6) Date; 7) Number of phages per 1 ml; 8) Water temperature; 9) Water level; 10) Cloudiness; 11) Normal; 12) Increase; 13) Cloudy; 14) Clear; 15) Semi-cloudy; 16) Decrease; 17) Stagnant water.

Table 120

Occurrence Process at the Investigation Points for Occurrence (1960, Takada Municipality)

① 調査場所 ② 月日	A 稲田立町			B 戸野目			C 四ヶ所			D 茨沢			E 鴨島			⑦ 平均 株茎数
	発病 株数 ④	発病 茎数 ⑤	発病 率 ⑥%	発病 株数 ④	発病 茎数 ⑤	発病 率 ⑥%	発病 株数 ④	発病 茎数 ⑤	発病 率 ⑥%	発病 株数 ④	発病 茎数 ⑤	発病 率 ⑥%	発病 株数 ④	発病 茎数 ⑤	発病 率 ⑥%	
6. 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.7
25	0	0	0	0	0	0	0	0	0	1	2	0.06	0	0	0	15.9
7. 11	0	0	0	0	0	0	0	0	0	1	2	0.04	0	0	0	25.0
25	2	2	0.09	0	0	0	0	0	0	8	24	1.03	3	4	0.17	23.2
8. 5	7	10	0.5	1	1	0.05	0	0	0	13	47	2.2	3	4	0.19	21.3
9. 2	8	19	0.9	1	1	0.05	0	0	0	15	63	3.26	13	31	1.55	19.3
16	10	29	1.5	1	1	0.05	0	0	0	18	133	6.89	20	69	3.57	—

Note: Figures in the table are values per 100 stems.

[Legend]: 1) Place of investigation; 2) Item; 3) Date; 4) Number of diseased stems; 5) Number of diseased stalks; 6) Rate of diseased stalks; 7) Average number of stalks per stem; A) Inada Tate-cho; B) Tonome; C) 4 places; D) Ibarazawa; E) Kamojima.

Especially in the summer cessation period in rice in mid-August, the number of phages declined. After continuous rain from 7 September on, occurrence showed considerable progress in the late-maturing varieties after mid-September. During this period a considerable number of phages was again detected. Thus phage fluctuations agreed with the progress of occurrence.

4. 1961 Test

1) Test Method

Same as 1960.

2) Test Results and Observations

Test results are shown in Table 121.

Table 121

Relation Between the Fluctuations in the Amount
of Phage in Rivers and Water Channels
and Occurrence (1961)

場①所 ⑤事項 ⑥月日	②荒川				③大道用水				④子安用水			
	ファージ 量 1ml 当り ⑦	水温 (°C) ⑧	水位 ⑨	濁度 (°) ⑩	ファージ 量 1ml 当り	水温 (°C)	水位	濁度	ファージ 量 1ml 当り	水温 (°C)	水位	濁度
4. 6	0	5.5	増⑪	濁⑫	0		減	濁	0		平	透
13	0	8.0	平⑬	"	0	17.5	"	半濁	0	15.5	"	半濁
20	0	9.0	"	"	0	10.5	増	濁	0	9.0	増	減
27	0	7.0	増	"	0	9.0	"	半濁	0	8.0	"	透
5. 4	0	11.5	平	半濁⑭	0	12.0	"	"	0	12.0	平	半濁
11	0	12.0	"	"	0	17.5	減	透	1	14.5	"	"
18	0	13.5	減⑮	透⑯	0	19.5	"	"	2	14.5	減	透
25	0	18.5	"	"	2.5	15.5	増	濁	9	17.0	増	濁
6. 1	2	16.0	"	"	6	16.5	"	半濁	10	19.0	平	半濁
8	0	21.0	"	"	0	23.5	減	透	0	20.0	増	"
15	0	21.0	"	"	2	17.0	平	半濁	4	19.0	平	"
22	18	24.0	"	"	69	20.0	増	"	44	22.0	"	"
29	39	17.0	増	濁	153	19.0	"	濁	412	21.0	増	"
7. 6	120	21.0	減	半濁	330	21.0	平	"	824	29.0	減	透
13	4,680	21.0	平	濁	4,630	22.0	減	"	12,320	26.5	"	濁
20	7,400	24.0	減	半濁	5,520	22.0	増	"	4,800	25.0	平	半濁
27	3,200	28.0	"	透	6,440	28.5	"	半濁	425	29.0	"	"
8. 3	5,840	26.5	"	半濁	7,520	28.0	"	"	7,200	28.0	"	"
17	7,000	27.0	"	透	10,560	25.0	"	"	7,800	27.0	"	"
31	430	26.0	"	半濁	895	23.0	平	"	890	24.0	"	"
9. 14	70	25.5	"	透	37	30.0	減	透	1,180	28.0	減	透
29	20	22.0	"	"	90	21.0	平	半濁	3,240	25.0	"	"
10. 12	486	16.0	増	濁	850	18.0	"	"	1,300	19.5	平	半濁
26	11	14.5	減	半濁	110	15.5	減	透	24	15.5	減	透

- ⑪発生経過
⑫6月14日 サヤスカグサ発病発見
⑬7月5日 試験場内サヤスカグサ発病、稲の初発を認める。(育種圃場)
⑭7月13日 全般初発生
⑮8月2～3日の大雨(110mm)で8月中旬かなり発病した。
⑯しかし、8月下旬～9月にかけて好天であつたため、発病は局部的で、病勢の進展は緩慢であつた。全般的に発生の少ない年であつた。

Note: H5820 (type A) was used as the indicator strain for the phage detection.

Legend: 1) Place; 2) Arakawa; 3) Daido Channel; 4) Koyasu Channel; 5) Item; 6) Date; 7) Number of phages per 1 ml; 8) Water temperature; 9) Water level; 10) Cloudiness; 11) Increase; 12) Cloudy; 13) Normal; 14) Semi-cloudy; 15) Decrease; 16) Clear; 17) The process of occurrence; a) The occurrence of the disease in Leersia oryzoides (Linn.) Sw. on 14 June; b) On 5 July the occurrence in Leersia oryzoides (Linn.) Sw. within the station and the first occurrence in rice were observed; c) On 13 July the first general occurrence; d) Because of the heavy rain on 2-3 August (110 mm), considerable occurrence in mid-August; e) But because the weather was fine in late-August to September, occurrence of the disease was locally limited, and the progress of the disease was slow. It was a year of generally small occurrence.

In 1961, phages were detected from 11 May in a branch of the small Koyasu channel; from 25 May in the Daido channel; and only in June in Arakawa. Comparison with 1958 and 1960, which showed medium and small occurrence, shows that this was later than in those years or at the same time.

It is noteworthy for 1961, that first detection was not at the same time in the three places. Detection was early in the Koyasu channel which had abundant Leersia oryzoides (Linn.) Sw. and nurseries, but it was late in the Daido channel and Arakawa which were wide and had large amounts of flow. In this investigation the frequency of measurement per month was more than in the three preceding years. Thus a more clear picture was obtained. In Arakawa, 2-3 ml of phage was detected on 1 June, but detection was unstable until mid-June, and the time of detection was later than usual. The cause of this, was probably because the time of thawing was delayed because of the largest snowfall in several years, with maximum accumulated snow of 168.5 cm, 98 days of snow and a low mean temperature in March (lower by 2.8°C). Therefore bacterial multiplication was suppressed in Leersia oryzoides (Linn.) Sw., and the general time of phage discharge into irrigation water was delayed. However, after mid-June, scores of phage per 1 ml were detected at all places.

Especially in the vicinity of the Koyasu channel, on 22 June, (44/ml), occurrence in Leersia oryzoides (Linn.) Sw.

was observed. And, on 6 July when the amount reached 800/ml, the first occurrence in rice was observed in nearby paddy fields. The relationship between the number of phages detected and the time of first occurrence agreed with past results.

Thereafter on 13 July, 10^4 /ml of phage was detected in the Koyasu channel, and infected fields were observed at several places. The sudden increase in the number of phages detected at this time was probably because samples were collected at a point where drainage from infected fields directly flowed into. This was valid in the case of the number of phages detected in the Daido channel and in Arakawa. In both places, 4,000 phages were detected, and this seemed to indicate a trend reflecting occurrence in rice at this period. This trend continued until mid-July and mid-August. Thus with the increase of infected fields nearby, the number of detected phages also increased. The number began to decline gradually in late-August, and this corresponded to the stagnation and end of occurrence in the latter half period of rice growth in this year.

5. Summary

The results of four years of investigation, from 1958 to 1961, are summarized in Tables 122, and 123. That is:

1) Phage fluctuations in rivers and channels correspond to the increase of infected fields, the degree of occurrence, the progress and cessation of occurrence, thus, indicating a close mutual relationship.

2) During the summer cessation period, there was a temporary decline in the number of phages detected in rivers and reservoirs.

3) There was no increase or decrease in the number of phages after the discovery of infected paddy fields, preceding the progress and stagnation of disease.

4) However, the first detection of phage tended to be late in 1958, 1960, and 1961 when the occurrence was late, and quite early and definite in 1959, a year of early and much occurrence. The time of the first detection of phage seemed to be related to the February-April temperature of the year.

5) In the relationship between the number of phages and occurrence, when the number of phages was over 100 per

1 ml in water channels, first occurrence was observed in nearby paddy fields. When it was over 1,000, the first stage of general occurrence set in.

6) From the above results, it seems that the time of occurrence in an area or the degree of occurrence can be forecast by measuring the number of phages in water channels or in rivers where irrigation from the infected area flows in and out.

Table 122

Phage Fluctuation in Rivers and Water Channels (1958-1961), per 1 ml of Irrigation Water

① 調査場所 ② 年月日	③ 荒川				④ 幹線用水 (大道用水)				⑤ 小用水路 (子安用水)			
	1958	1959	1960	1961	1958	1959	1960	1961	1958	1959	1960	1961
④ 4月	上旬⑦		0	0			0	0	0	1	0	0
	中⑧		0	0		0	0	0		2	0	0
	下⑨		0	0		1	0	0		6	0	0
5月	上旬	0	0	0		5	0	0	0	21	0	0
	中	0	3	1		2	1	0	6	4	3	1
	下	3	0	1	1	3	7	2	6	2	9	9
6月	上旬		7	4		8	7			19	11	10
	中	17	10	18	46	21	29	2	4	17	24	4
	下		95	31		93	85	69		73	16	44
7月	上旬	19	130	18	36	420	169	330	45	148	90	824
	中	275	415	345	395	495	900	4,630	260	860	170	12,320
	下	343	2,890	2,300	2,100	2,140	6,750	4,880	3,560	1,810	1,500	4,800
8月	上旬	1,600		5,150	1,740		4,600	7,520	4,520		2,600	7,200
	中	76,000	260	300	92,000	1,170	500	10,560	20,000	680	750	7,800
	下	6,100	1,390	1,200	430	12,200	2,400	1,100	880	16,000	2,040	3,000
9月	上旬	1,300	1,150	170	3,300	1,570	550		159,000	3,710	620	
	中	4,300	30	4,790	70	3,000	20	1,200	37	3,500	1,510	1,180
	下	600	505		20	1,200	3,345		90	400	2,200	3,240
10月	上旬		15	50		70	35	850		160	40	1,300
	中	3,200	22		14,500	134	16		1,300	350	65	
	下				11	7		110		24		24

Note: Lyso-type A was used for phage detection. (1958-1959 Shinjo strain, and 1960-1961 H5820 strain)
Figures with dotted lines underneath show the number of phages at the time of the first occurrence. — shows the number of phages at the time of the first occurrence in rice.

[Legend]: 1) Place of investigation; 2) Arakawa; 3) Main water channel (Daido Channel); 4) Small water channel (Koyasu channel); 5) Date; 6) Month; 7) First ten days of month; 8) Middle ten days of month; 9) Last ten days of month.

Table 123

Summary of the Tests on the Relationship Between the Phage Fluctuation in Rivers and Channels and Occurrence (1958-1961)

① 年次	② フェージ初検出月	③ 2~4月中の平均気温・°C	④ ヤマスカグサ初発月日とフェージ量	⑤ 稲初発生期とフェージ量	⑥ 夏期フェージの減少	⑦ 8月の気温・°C ⑧ 前半 ⑨ 後半	⑩ 被害多品種	⑪ 被害程度
⑬ 1958年 ⑭ 平年発生	5月26日	9時 ⑮ 6.9 max 10.8 min 5.3	6月27日 17~46/ml	7月16日 260~395/ml	なし ⑯	28.8 24.6	⑰ 晩生	⑱ 少~中
⑬ 1959年 ⑭ 早・多発年	4月23日	9時 8.6 max 12.1 min 3.0	6月21日 10~21/ml	7月2日 73~95/ml	あり ⑯	29.5 25.5	⑰ 早生	⑱ 多
⑬ 1960年 ⑭ 遅・少発年	5月21日	9時 6.8 max 11.2 min 2.0	7月10日 18~169/ml	7月18日 170~900/ml	あり	30.5 26.7	⑰ 晩生	⑱ 少
⑬ 1961年 ⑭ 平年~少発年	河川 ⑮ 6月1日 用水路 ⑮ 5月11日	9時 6.1 低 max 9.7 温 min 0.7	6月14日 0~4/ml	7月5日 120~824/ml	なし	27.3 27.3	⑰ 早生	⑱ 少

[Legend]: 1) Year; 2) Date of the first phage detection; 3) Average temperature of Feb.-Apr; 4) Date of the first occurrence in Leersia oryzoides (Linn.) Sw. and number of phages; 5) First occurrence in rice and number of phages; 6) Decline of phages in summer; 7) Temperature in August; 8) First half; 9) Latter half; 10) Much injured varieties; 11) Degree of injury; 12) Usual occurrence; 13) Early and much occurrence; 14) Later and small occurrence; 15) Usual and small occurrence; 16) Usual; 17) Higher temperature; 18) Low temperature; 19) Late maturing; 20) Early maturing; 21) Small to medium; 22) Much; 23) Small; 24) Rivers; 25) Channels; 26) 9:00 hour; 27) positive; 28) negative.

Section 5. Several Problems in the Measurement of Phages in Irrigation Water

There are several problems in collecting indicator strains and samples for the quantitative phage test in irrigation water. The most important among them is phage fluctuation due to the time of collection of irrigation water, the amount of flow, and the presence of precipitation. In other words, the dependability of measured values as representative values is low. In the following, along with the problems of selecting indicator strains for the detection of phage, a few problems concerning the sampling of irrigation water are reviewed.

1. Selection of Indicator Strain for Detection

As has already been described, phages of the pathogen of rice bacterial leaf blight OP_1 , OP_{1h} , OP_{1h2} , OP_{1t} , OP_2 , and OP_{2m} have been isolated. Consequently, it is necessary to know the distribution of phages in the area under investigation before the quantitative phage test, because it differs according to areas. As has been dealt with in Section 1, Chapter VI, OP_1 , OP_{1h} , and OP_2 are widely distributed in the Hokuriku District. Therefore, the quantitative test can be well effected by selecting Typa A and B bacteria, which have phage affinity. But as has been clarified by the tests mentioned in Section 3 and 4, as a practical matter, only with Type A bacteria can the fluctuation of phage can be traced in many areas.

However, in a case like Okawa Municipality, Fukuoka Prefecture where only OP_{1h} is distributed, it is necessary to investigate the kinds and distribution of phage in the area and then to select suitable strains (Lyso-types).

Table 124 indicates the results of the investigation on the phages detected at the Hokuriku District Agricultural Experimental Station at Takada Municipality, Niigata Prefecture; Aono-Jumonji, Naoetsu Municipality; Nanuka-cho, Nagasaki Municipality, and one more place, Makicho, Nishi-kamabara-gun, and Niitsu Municipality.

Lyso-types A, B, C, D and E were used for the experiment in Table 124. From the host range of phage as has been described, lyso-type A has affinity with $OP_1 + OP_{1h2} + OP_2$; lyso-type B with $OP_{1h} + OP_{1h2} + OP_2$; lyso-type D with $OP_{1h2} + OP_2$; and lyso-type E with OP_2 (lyso-type C is phage-resistant). Therefore, the amount of OP_1 and OP_{1h} can be computed by deducting the amount of OP_{1h2} and OP_2 measured

Table 124

Relationship Between Indicator Strains for Detection
and the Number of Phages

Place	Year	Date	Indicator Strain for Detection			
			Shinjo Strain (Type A)	Benikonaya Strain (Type B)	H5925 (Type D)	H5913 (Type E)
Kaminada, Takada Municipality	1959	11 September	933	74	77	69
Aono-Jumonji, Naoetsu Municipality	1959	2 August	11,400	224	231	159
Nagakura-cho, Nagaoka Municipality	1959	9 July	1,920	2,150	--	2,850
Nanuka-cho, Nagaoka Municipality	1960	10 July	9,100	900	820	700
Maki-cho, Nishi- Kamabara-gun	1960	12 August	461	720	106	51
Ozeki, Niitsu Municipality	1960	12 August	333	38	22	17
Otagawa, Nagaoka Municipality	1961	19 July	600	223	138	67
Zendoji, Nagaoka Municipality	1961	19 July	388	15	8	13

Note: H5839 (Type C) was also used for experiments, but no plaque reaction was produced.

by Lyso-types D and E from the amount of phage detected by lyso-types A and B. However, whether or not the amount of phage by groups can be computed by the foregoing mechanical computing method must be given further examination, because there seems to be mutual interference between OP_1 , OP_{1h} , and OP_{1h2} and the bacteria with affinity to the former. Yet, if we assume the aforementioned relationship exists and study the results shown in Table 124, the majority of phages in Niigata Prefecture are OP_1 and OP_2 , and sometimes, depending on areas, OP_{1h} and OP_{1h2} are detected. However, the geographical distribution of these phages is not clear. There will be a need for further examining indicator strains if new groups of phage should be detected in the future. However, the past tests show that the phage fluctuations in irrigation water can be systematically investigated by using lyso-types A, B, and E for the quantitative phage test.

2. Phage Fluctuation in One Patch of Paddy Field

1) Test Method

As shown in Figure 27, irrigation water was collected at several places within one patch in the Hokuriku District Agricultural Experimental Station, and the number of phages per 1 ml was quantitatively tested. The measurement of phage was according to the plaque computing method, and H5820 (type A) was used as the indicator strain. The variety of rice grown in paddy fields was Ginmasari, and irrigation water was collected at 10:00 on 11 July.

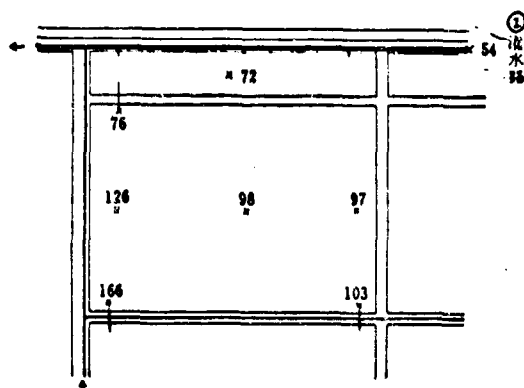


Fig. 27 Number of Phages in One Patch (per ml of Irrigation Water)

[Legend]: 1) Irrigation water channel.

2) Test Results and Observations

As shown in Figure 27, the number of phage per 1 ml of surface water in one patch was 76-166, with fluctuations in measured values. In this test, water was collected while irrigation was progressing. There were less phages at the entrance than at the exit.

3. Phage Fluctuations in Paddy Fields with Constantly Flowing Irrigation Water

1) Test Method

As shown in Figure 28, phage fluctuation in three adjoining patches of paddy fields of about 3-5a each were investigated when irrigation water channels, entries and drainage exits were alternated. Sample water was collected at 10 a.m., and Shinjo strain (lyso-type A) was used as the indicator for the detection of phages. The test took place on 11 August 1960.

2) Test Results and Observations

Test results are shown in Figure 28.

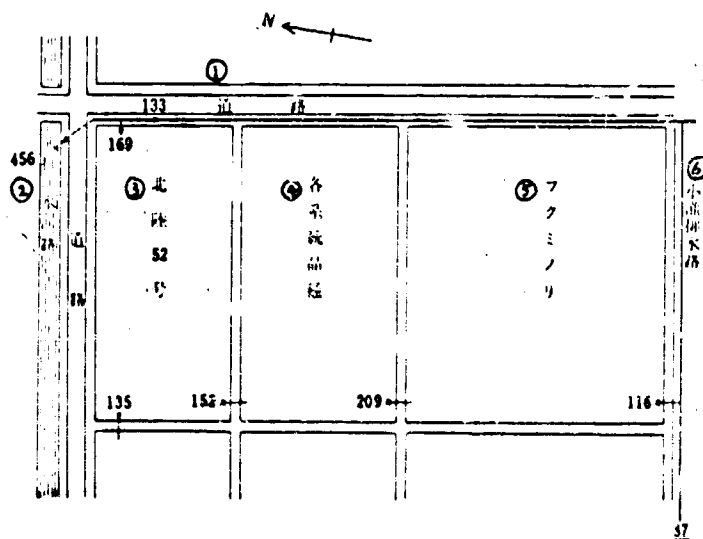


Fig. 28 Phage Fluctuation in Paddy Fields
(Number of phages per 1 ml of
irrigation water)

[Legend]: 1) Road; 2) Drainage channel; 3)
Hokuriku No 52; 4) Various varieties; 5)
Fukuminori; 6) Small irrigation water chan-
nel.

As shown in Figure 28, there is a tendency toward fewer phages at points closer to the source of irrigation water in paddy fields. In the present survey, the number of phages in the surface water was 116-209 per 1 ml, without too much fluctuation; however the number of phages in irrigation channels was 87/ml. From this, it is estimated that in paddy fields where water is directly fed in from irrigation channels, there is phage fluctuation in one patch. Consequently, it was considered necessary to mix samples collected at two or three places for phage measurement.

4. The Time of Phage Fluctuation in Irrigation Water

With the irrigation channels, drainage ditches, reservoirs, paddy fields, and Daido channel (already referred to) in the Hokuriku District Agricultural Experimental Station as objectives, chronological phage fluctuation produced by the different time of collection was studied.

1) Test Method

Shinjo strain (type A) and the plaque computing method were used. During a 24 hours period from 15:00 hours 17 June 1959 to 15:00, 18 June, irrigation water was collected every three hours for investigation.

2) Test Results and Observations

Test results are shown in Table 125.

As shown in Table 125, there were changes in the number of phages detected according to the time of collection of irrigation water. Fluctuation was great in the terminal small irrigation channel branching out from the main water channel.

Also there was fluctuation even in the paddy fields, drainage ditches, and reservoirs where there was no movement of irrigation water during the investigation. No phages were detected in the paddy fields which were dried up due to droughts. Since fluctuation was smallest in the Daido channel which was very wide and had much flow (3 m), these factors must be kept in mind in selecting the method and places of collection. In short, it was determined that the number of phages fluctuated greatly depending on the time of collection, and this acted as a factor for disturbing measured values.

Table 125
Chronological Change of Phage in Irrigation
Water (per 1 ml)

Date	Time	Paddy Field		Koyasu Channel**		Drainage Ditch	Pond	Daido Channel
		1	2*	1	2			
		Hour						
17 June	15	12	0	17	21	21	21	21
	18	24	0	14	32	150	12	9
	21	20	0	21	23	148	13	12
18 June	0	6	0	12	26	104	5	19
	3	2	0	159	7	118	12	25
	6	24	1	77	11	71	12	24
	9	2	0	41	0	45	11	29
	15	14	0	3	1	8	2	9

Note: * No water during droughts. Water produced by footprints was collected.

**Branch irrigation water channel.

5. Chronological Change of Phage in Water Channels and Irrigation Channels

In the test described in the preceding section, it was discovered that there were changes in the number of phages detected according to the time of collection of irrigation water, and this affected the reliability of the data for pursuing phage fluctuation. Therefore, the chronological change in the number of phages measured according to the width of rivers, water channels, and the amount of their flow was examined.

1) Test Method

During a 24 hour period, from 0900 30 June 1961 to 0900 1 July 1961, irrigation water was collected every three hours at the rivers, water channels, and irrigation channels flowing through the vicinity of the Hokuriku District Agricultural Experimental Station. The number of phages was measured and compared according to the plaque computing method. H5820 (type A) strain was used as the indicator strain.

2) Test Results and Observations

Test results are shown in Table 126.

As shown in Table 126, the chronological phage changes in rivers, water channels, and irrigation channels were great in places of narrow widths and small amounts of flow as in small irrigation channels, and drainages; in other words, in places subject to the daily changes in amount of flow. However changes were slight in rivers with wide widths and where the daily change in the amount of flow was slow. It was also observed that there were rather great chronological changes in paddy fields.

From the foregoing results, it seems necessary when collecting test samples from small irrigation water channels or paddy fields where the chronological changes in the number of phages are great, to collect several samples to quantitatively test for phages each time, and thus find their mean values, or quantitatively test for the number of phages after mixing irrigation water samples collected at different hours.

Table 126
Chronological Phage Changes in Rivers, Channels,
and Drainage Ditches

Rivers and Water Channels	Time River width hour	In the Hokuriku District Agri- cultural Experimental Station				
		Arakawa Channel	Daido Channel	Koyasu Channel	Irrigation Channel	Drainage Channel
Date		34 m	3 m	90 cm	40 cm	55 cm
30 June	9	13	22	55	34	13
	12	13	14	12	4	71
	15	7	22	26	5	56
	18	14	18	10	8	6
	21	20	22	4	5	12
1 July	0	24	26	2	3	7
	3	17	7	39	45	23
	6	23	6	20	56	60
	9	18	8	24	32	14
						11
						0
						2
						22

6. Viability of Phages in Irrigation Water

If samples must be collected several times in one day as described in the preceding section, phage may die during storage due to the impact of high temperatures and microbes in irrigation water, depending on the time of collection. In this test, the process of dying and the decrease of phage in irrigation water during storage was investigated, and the impact of temperature on the storage of test samples from irrigation water was examined.

Table 127

Viability of Phages in Irrigation Water (per 1 ml)

Storage Temperature °C	Time at Collection	Elapse of Time After Collection of Water								
		Hour						Day		
		24	48	72	7	8	9	13	25	36
30	387	186	121	86	22	6	6	7	0.6	0.2
25		208	102	88	4	5	2	0	0	0
5		340	362	51	42	12	13	4	3.3	2.1
Room Temperature 26-36°C		191	110	85	26	6	12	0	0	0

Note: Mean values gained from three repetitions.

1) Test Method

The test was conducted according to the method described in 5. The phages in irrigation water were OP₁ and OP₂.

2) Test Results and Observations

The viability of phages in irrigation water collected in the irrigation channels in the Hokuriku District Agricultural Experimental Station are shown in Table 127.

As shown in Table 127, when irrigation water is stored under at 26-36°C, the number of phages decreases in 24 hours to less than 1/2, in 48 hours to 1/3, and in 72 hours to 1/4. The incline of decrease is logarithmic. In contrast to this, phages stored in a refrigerator at 5°C, showed almost no decrease in 48 hours, but a rapid decrease in 72 hours. Thereafter, a similar trend as under other

temperature conditions was shown.

7. Summary

The results on the examination of the several problems presented in the quantitative test for phages in surface water, irrigation channels, water channels, and rivers are summarized as follows:

1) Indicator strains used for phage detection must be examined beforehand as to their affinity with other groups of phages distributed in the area under investigation.

2) When lyso-type A, B, and E strains are used, the quantity of phages by groups can be measured.

3) The number of phages in one patch of paddy field can fluctuate depending on the locations of collection. Therefore, it is advisable to collect samples from several places and mix them for the quantitative test.

4) The number of phages in irrigation water fluctuates according to the time of collection, and this trend is strong in the case of collection from places with narrow widths and small amounts of flow.

5) Therefore, it is desirable to collect several samples a day in measuring phage in surface water, irrigation channels, and water channels, and mix them for the quantitative test.

6) Test sample irrigation water can be preserved in a refrigerator at 5°C for up to 48 hours without decreasing the number of phages.

CHAPTER XIII. DISCUSSION AND CONCLUSION

There has been a gradually increasing trend in recent years in the occurrence and distribution of rice bacterial leaf blight in Japan. Especially of late, this has spread to Northern Japan and its damage is increasing. Despite research for the control of this disease by agencies throughout Japan, at present there are only a few ways of alleviating damage and of avoiding damage by using resistant varieties or by a few ways of cultivation and planting control. No chemical control has been found yet. Therefore, it is desirable at the present stage to make a preliminary clinical diagnosis to discover the occurrence of this disease as early as possible, to preliminarily diagnose the disease, and to properly treat the disease. In this study, from the aforementioned standpoint, the occurrence of rice bacterial leaf blight, especially its symptoms, the characteristics of its pathogen and phage, the infection process from overwintering through primary infection to secondary infection, and fluctuations in occurrence were studied and surveyed from various viewpoints, in order to find standards for diagnosis of the occurrence of this disease.

To begin with, the past idea of diagnosis was to inspect infected plants with precision, classify symptoms, determine proper nomenclature, isolate and identify pathogens, and inspect and diagnose the disease. For this, such methods as the discrimination of pathogens, diagnosis of symptoms, analytical diagnosis, physio-chemical diagnosis, serological methods, culture diagnosis of pathogens, and environmental occurrence diagnosis were used. Of these discrimination of pathogens with microscopes, symptom diagnosis, and environmental occurrence diagnosis are the usual methods adopted in searching for the causes of the diseases. Especially symptom diagnosis, is normally used as the simplest diagnosis method. However, all these are diagnostic methods designed for diagnosis after the infection of diseases. In contrast to this, in the present research the possibility of forecast diagnosis has been examined by examining the inoculation test for symptoms, pathogens, and phages not only from the viewpoint of mere observation and survey, but also from the aspect of utilizing the diagnosis, by applying the quantitative phage test to test samples in fields, by researching the overwintering of the pathogen,

the infection, latency, multiplication in rice, by quantitative phage testing in waters in infected areas, and thus by estimating indirectly the bacterial multiplication and occurrence.

In the following, based on the accomplishments of the present study, the standards for the diagnosis of the occurrence of rice bacterial leaf blight and observations concerning it will be described with some discussion.

1. Environmental Diagnosis

In any disease, the relationship between occurrence and environment, especially conditions of location and climatic conditions is surveyed, and then those conditions closely related to occurrence are pointed out and stressed as causal factors. The reason that the author has set up diagnostic standards for environmental occurrence is from the consideration that listing causal factors in the case of rice bacterial leaf blight in the constantly occurring zones is necessary for diagnosis.

As has been clarified from the test and results of investigation carried out by many researchers and the author, the following eight items are mentioned, because they are the necessary items of investigation for diagnosing the environment of the occurrence field as a whole before going into individual diagnosis. Of these, the growth and occurrence of Leersia oryzoides (Linn.) Sw. would be an indispensable item of investigation, because many instances point to it as the powerful source of primary infection in the constantly infected areas. It is confirmed by Tagami, et al¹²³ that in warm areas, the overwintering and survival of cut rice stems facilitates pathogen overwintering, and immersion, submersion, and flooding of rivers which assist the spread of the pathogen are closely related to rainfall and typhoons. Therefore, investigation of their timing and degree is necessary for analyzing the causes of occurrence.

That the relationship with water is specially taken up as an objective of diagnosis pertaining to the whole environment seems to be only natural because of the characteristic of this disease which is an infectious disease spread by water. From the above, the following items are mentioned as standards for diagnosing environment:

- 1) To determine whether the infected field is low and wet, a caved-in field, or on the perimeter of rivers and lakes.

2) The natural growth distribution and occurrence of Leersia oryzoides (Linn.) Sw. and other intermediate host plants.

3) The overwintering and survival of cut rice stems.

4) Water rising and flooding of rivers and lakes.

5) Precipitation (time, and volume, especially investigation at the time of nurseries, and the early and intermediate period of paddy fields is important.)

6) Investigation of typhoons (time, size, wind direction, wind velocity, whether or not they are rain storms)

7) Occurrence of the preceding year.

8) The fluctuation of temperature from January to April (for the estimation of the overwintering of bacteria).

2. Cultivation and Planting Control Diagnosis

It is needless to mention that the method of cultivation and planting influence the susceptibility of rice and the degree of occurrence. As far as this disease is concerned, there is only a little research and a few reports on the relationship between the cultivation and planting control and occurrence. However, through the investigation of the author, it was determined that occurrence after transplanting to paddy fields is increased by the forms of nurseries, and that water control (the presence of immersion and submersion) is strongly related to the degree of occurrence through the investigation of fluctuation of occurrence in nurseries and paddy fields and pathogen fluctuation. Consequently, investigations of these items seem to be necessary for diagnosis. The cultivation of infectious varieties, excess use of nitrogenous fertilizers, and early water draining are generally known as the cause of frequent occurrences. Therefore, these should be naturally added to the standards for diagnosis.

From the foregoing, the following items are regarded as contributing to diagnosis in the aspect of the cultivation and planting control:

1) Cultivated varieties.

2) Forms of nurseries and the location of nurseries.

3) Water control in nurseries (whether it was

deep-water control or not.)

4) Amount of fertilization (especially the time and amount of nitrogenous fertilizers)

5) Water control in paddy fields (presence of immersion and submersion, summer droughts, and early water draining.)

3. Diagnosis of Symptoms

1) The author classified the symptoms of rice bacterial leaf blight morphologically into the following four types to serve as the standards for diagnosis.

a) Rapid wilting type, b) Leaf edge type, c) Streak type, d) Spot type.

Of the above, the wilting type is a symptom observable in comparatively young leaves until tillering, and it is a symptom that occurs by clogging vessels with bacterial ooze of the pathogen multiplied in vascular bundles. Wilting is a symptom commonly observable in vascular diseases of other crops, but in rice, wilting seldom takes place because the silication of leaf tissues progresses after development of leaves or ear formation. This symptom is not regarded as being too important, but infected fields with this symptom tend to suffer severe damage.

2) As a mark shown by the pathogen itself, bacterial ooze or exudate formed on infected leaves serves as an important clue for early diagnosis during the latent period and for distinguishing from other similar diseases.

3) The simplified diagnostic method of diseased leaves (See Section 3, Chapter IV) is helpful, as the author has often experienced, for distinguishing similar diseases to rice bacterial leaf blight, and for the early discovery of the disease in the early period of paddy fields. This method seems to be applicable for fields since it does not require special instruments.

4. Identification of the Pathogen and the Diagnosis of its Pathogenicity

1) Application of the method of utilizing affinities (plaque reaction) peculiar to different groups of OP₁ and OP₂ phages for identifying the pathogen.

2) The application of the method of classifying the

pathogens of rice bacterial leaf blight by the host range of the OP₁ and OP₂ group phages into the five lyso-types of A, B, C, C, and E.

The example of such application of phage to crop diseases has begun with the identification of the wilting bacteria of corns by Thomas.¹³⁰ However thereafter, Thornberry, et al¹³⁰ used the peach boring bacteria phage for the identification of *X. pruni*. Katanelson, Sutton, Bailey, et al^{47,48,116} devised the live bacteria detection method which uses phages that have peculiar affinities for the diagnosis of the yellow spot pathogen and the leaf burning pathogen of kidney bean, and proved that bacteria overwinter and survive in the seeds collected in the preceding year from infected stems.

The idea of utilizing the close relationship between phage and bacteria for diagnosis is very good as long as the object is a bacterial disease.

3) The pathogenicity of isolated strains are classified by needle inoculation detection of rice varieties for determination into the following three groups.

Group I. (Strong): Strains that show strong pathogenicity against susceptible varieties (Jugoku, Takara, and Kamiyama) and also against resistant varieties (Ogyoku, Zensho No. 26, Akajinriki, Kamizeki No. 1).

Group II. (Medium): Strains that have strong pathogenicity against susceptible varieties, but are weak against resistant varieties.

Group III (Weak): Strains that have weak pathogenicity even against susceptible varieties.

At first, research revealed that there was no relationship between lyso-types and pathogenicity. Therefore, attempts were made to classify, according to the inoculation reaction of bacteria, varieties with different resistance, as was done in the race of rice blight bacteria, and the aforementioned standards were obtained. There is no effective control chemical against the pathogenicity of this pathogen, so the cultivation of resistant varieties constitutes the pillar of control. Certain resistant varieties will not do in areas where much of Group I strains is distributed. It is desirable to raise wet rice varieties that show strong a resistance reaction to Group II strains as well as to Group I strains.

5. Disease Diagnosis (Diagnosis of the amount of bacteria contained)

Wakimoto and Yoshi¹³⁸ devised a method for quantitatively testing bacteria using the technique of the one-stage multiplication test of *X. oryzae* phage, and established a method which has more precision than the previously published live bacteria detection method. Wakimoto¹³⁷ proved by this, the overwintering and survival rice bacterial leaf blight in diseased leaves and seeds.

This quantitative test of bacteria has an excellent point in that it can quantitatively test the amount of bacteria contained in plants. In the present study, this method has been applied in searching for the occurrence from the overwintering of this pathogen to primary infection and to secondary infection. In other words, by the quantitative test method of bacteria the fluctuation of pathogen multiplication in rice and irrigation water can be ascertained. Based on the investigation of the fluctuation of the occurrence of the disease conducted at the Hokuriku District Agricultural Experimental Station and the tests made at the Kyushu District Agricultural Experimental Station on the life cycle of the pathogen on rice leaves, the following standards for diagnosing the disease concerning infection, latency, and multiplication have been arrived at:

1) Although no occurrence is observed in nurseries, there is still a great hazard of infection in seedlings even in the Hokuriku District.

2) The Shifts in the amount of bacteria in nurseries is not constant, and the amount varies with the locations of nurseries, the density of growth of the intermediate host plants of the pathogen around nurseries, and the presence and frequency of immersion and submersion due to rainfall.

3) The fluctuation in the amount of bacteria on rice leaves in paddy fields form a characteristic curve. In other words, there are two times when the curve forms peaks. The first peak is the first period of increase during the early period of occurrence observable in the intermediate tillering stage, and the second peak shows the second period of increase when bacteria multiply and are spread by typhoons after the young ear formation period. Between these peaks, there is a trough called the summer stagnation period when the amount of bacteria decreases due to the summer high temperature and dryness, and the force of the disease also comes to a standstill.

4) The increase in the amount of bacteria in the first period of increase is strongly influenced by the amount of bacteria in or by the amount of infected bacteria in the early period of paddy fields, and in this manner the degree of occurrence is controlled.

5) While a seedling containing bacteria immersed once in the suspension with more than 10^3 /ml concentration shows occurrence in one month, even if it is soaked in suspension with a 10^8 /ml concentration, the maximum rate of occurrence in stems is 8.5%. However, those seedlings which are given several immersion inoculations in the latter half of nursery and thus given the latent period for multiplication in seedlings, show severe occurrence after transplanting to paddy fields. (This shows the importance of the infection frequency, infection period in nursery, and latent multiplication in seedlings.)

6) From late July to early August, after the first period of increase, the amount of bacteria on rice leaves show a temporary decline, and the disease comes to a standstill (summer stagnation period). This seems to be due to the high temperature and dryness of the time.

7) The increase in the amount of bacteria in the second period of increase is closely related to the damage caused by the typhoons. In other words, bacteria are dispersed by typhoons as far as 64 m (in the case of 23 m/sec winds) and rice plants are damaged. Thus, typhoons have a direct and indirect impact on the spread and invasion of bacteria. For this reason, the amount of bacteria on rice leaves shows a great increase, and similarly as in the first period of increase, the amount of latent bacteria rises to 10^5 per leaf until occurrence, and then occurrence rapidly spreads.

6. Forecast Diagnosis.

Since phages in irrigation water are regarded to increase or decrease in relation to multiplication activity in rice, Leersia oryzoides (Linn.) Sw., the estimation of bacterial multiplication in fields may be possible from the time of detection and quantitative changes of phage. In the present test, phage fluctuation in irrigation water agrees with the occurrence process in rice plants, and based on the following standards, data for forecast diagnosis of the occurrence of the disease are obtained.

1) Diagnosis of the overwintering of bacteria:
Phages in rivers, water channels, and small irrigation

channels are quantitatively tested each week before the nursery period, and the time of the detection, fluctuations in detection, and in the amounts detected can be compared each year. By doing this bacterial overwintering and multiplication in the nursery period can be estimated.

2) Locality forecast diagnosis: When the measurement of phage in rivers and main water channels flowing through the area of occurrence is carried out periodically, from the early period of paddy fields, shows more than 100 per 1 ml of irrigation water, the first occurrence in rice plants in the area concerned is observable.

3) Regional or locality forecast diagnosis: The periodical measurement of phage in small water channels and irrigation channels from the early period of paddy fields reveals the following relationship between the number of phages detected and occurrence:

a) Less than 50 per 1 ml of irrigation water... the first occurrence in Leersia oryzoides (Linn.) Sw.

b) About 100 per 1 ml of irrigation water...10 days to two weeks before the first occurrence in rice plants.

c) 1,000-2,000 per 1 ml of irrigation water... the period when occurrence is discovered in the region concerned (village as the unit) or locality (= 100 ha.)

4) Point forecast diagnosis (several patches of nurseries or paddy fields)

a) Phages in irrigation water in nurseries are generally small (the number seldom passes 30 per 1 ml in the Hokuriku District). But when the amount detected is more than other places or in usual years, occurrence in paddy fields is frequent and the degree is severe.

b) Phages in the surface water in paddy fields are the amount of so many phages per 1 ml at the time of transplanting. This gradually increases. As an example, the following relationship between the number of phages detected in the intermediate tillering stage and occurrence is shown.

Less than 50	per 1 ml of surface water...	small occurrence
About 100	" " "	...medium occurrence
Over 10 ³	" " "	...much occurrence

c) When more than 10^4 phages per 1 ml of surface water of infected fields are detected, it serves to confirm the first occurrence.

The foregoing was an attempt to estimate the activity of the pathogen indirectly by the direct quantitative phage test under natural conditions. Although a simplified method of the aforementioned quantitative bacteria test has been devised,¹⁴² this still requires a high degree of skill in operation, instruments, and serum, and so makes it difficult to serve as a useful general diagnostic method. In this respect, the diagnostic method of directly measuring phages in irrigation water as advocated by Tagami¹²¹ is very simple in operation, even though it is an indirect method, and it allows for the detection of many samples in a short period of time. Thus, it is a highly applicable diagnostic method for practical use. This method, as Fulton²⁶ and Kelment^{54,55,56} recognized, is difficult to apply. However the phage of the pathogen of rice bacterial leaf blight has no parasiticity on the yellow plant pathophytes and it shows affinity peculiar to its group with the pathogen of rice bacterial leaf blight. Therefore, it seems that X. oryzoides phages in rivers and water channels in paddy field areas have close relationships with the multiplication and death of the pathogen of rice bacterial leaf blight.

The results gained by the investigation conducted by the author seem to serve as suitable analytic data for the occurrence process of each year, and also as the basis of grasping the fluctuations of the pathogen until occurrence and thereafter. That is, the life cycle of the pathogen can be regarded as overwintering in intermediate host plants -- multiplication in the following spring -- suspension in irrigation water -- rice infection -- latent multiplication -- occurrence -- secondary infection -- progress of disease -- damage -- end -- overwintering. If so, the fluctuation of phages in irrigation water is closely related to bacterial fluctuation, and furthermore to the fluctuation of the occurrence of this disease.

Consequently, the overwintering of the pathogen before occurrence, and the fluctuation of phages in grasses or rice during the latent multiplication period can serve as the basis for observation related to the forecast of occurrence of the disease thereafter. However, as was described in Sections 3 and 4, Chapter XII, phage fluctuation in waters during the second period of occurrence, show that the number of phages detected are not observable. Therefore, it seems to be difficult to use phage fluctuations

in water for the forecasting occurrence in the latter half of paddy fields.

Yet, according to the results of the investigation made by the author, if one selects the locations of the sample irrigation water, and quantitatively tests for phages three to six times a month throughout the year, and records their measured values for yearly comparison, after having examined the size of rivers and water channels, their routes, and locations in the infected areas, an estimation of the multiplication activity of the pathogen in the nursery and early period of paddy fields would be possible. Furthermore, the forecast of occurrence in the nearby areas would be possible. If this method is supplemented by instances of observation on points by applying it to the surface water of fields under observation with constant cultivation conditions, this will result in providing important diagnostic data for forecasting the occurrence of rice bacterial leaf blight in the year.

CHAPTER XIV. SYNOPSIS

This study has intended to clarify the ecology of rice bacterial leaf blight and to investigate and observe the symptoms of the disease, the morphology, pathogenicity of the pathogen, and the pathogen phage in order to contribute to establishing early forecasting and control methods for this disease. At the same time, the life cycle of the pathogen from overwintering to secondary infection through primary infection was analyzed, and diagnostic study on the relationship between the number of phages in irrigation water and occurrence of this disease were studied. Results obtained from these may be summarized as follows:

1) Common and popular names of rice bacterial leaf blight in Japan were surveyed and listed.

2) Description has been made on the history of occurrence of the disease in Japan, changes in the infected area, and especially of the occurrence in Northern Japan, in the Hokuriku District in particular. The remarkable damage caused by the occurrence of the disease in recent years in Northern Japan was noted, and the area of occurrence in the Hokuriku District was defined.

3) From observation of the symptoms of rice bacterial leaf blight, the symptoms can be classified into four types: wilt-type, edge-type, streak-type, and spot-type. And symptoms of each type were described. It was clarified that in the early period of growth, wilting peculiar to vascular diseases would appear, as a point to be watched for in its early discovery.

4) Diseases and insect damage similar to rice bacterial leaf blight and other symptoms were described. And in the aspect of disease and insect damage, damage caused by Leptosphaerella oryzae (Myake) Hara, Fusoma triseptatum Saccardo, virus, and Chilo simplex Butler, and damage by wind were pointed out.

5) As a discrimination method between leaves infected with this disease in its early stage of occurrence and leaves with similar diseases, the method of secretion in water of the bacteria multiplied in leaf tissues was devised.

6) Observations were made on the morphological characteristics of the pathogen of rice bacterial leaf blight with an electron microscope. The results show that cultured bacteria are rod or short rod shape, with a polar single flagellum, of 0.65×1.74 microns, and a capsule-like membrane of a thick slime layer were recognized. However, bacteria in host plants (bacteria in diseased leaves) are short rod or globular shaped, of a polar single flagellum, and measure 0.49×0.82 microns; but the existence of a capsule-like membrane is not clear. The latter is smaller by one size compared with the former. Also granular protuberances were observed on the latter's surface.

7) The results of the tests on the host range of the pathogen of rice bacterial leaf blight showed that in addition to the already known intermediate host plants, Leersia oryzoides (Linn.) Sw., Leersia japonica, Makino, Zizania latifolia (Turcz), and Phalaris arundinacea Linn., parasitic-ity by needle inoculation was clearly proved in Leersia oryzoides (Linn.) Sw. var. japonica (Rets) Honda, and also slightly with a low rate of 13.6% in Setaria viridis Beauv.

8) A new group of phages, OP₂, of the pathogen of rice bacterial leaf blight was isolated and identified.

9) The biological and physio-chemical characteristics of OP₂ were tested. This phage has a wider host range than other phages and a different serological reaction. Its 50% inactivation temperature was 62-64°C. In its one-step growth process in relation to lyso-types with affinities, its latent period was over 70 minutes, its rising period was over 40 minutes, and the average burst size was over 18.

10) The morphological observations of OP₂ phages by electron microscope revealed that the head was 70×70 m, its tail was 85 m, its thickness was 25 m. A marked difference from other known OP₁ phages in the shape of tail was noticed.

11) Among OP₂, those phages that form pin-hole plaques have roughly the same host range, serological reaction and morphological characteristics as OP₂, except their difference in plaque formation against lyso-type A bacteria, and the one-step growth process. However, they are markedly different from OP₁ or OP_{1h}, so they have been named OP_{2m} as plaque-shape mutants of OP₂.

12) By the discovery of a new OP₂ group phage, the pathogens of rice bacterial leaf blight can be classified

by their plaque reaction into four groups of phage, including other phages into the five lyso-types: type A, type B, type C, type D, and type E.

13) The investigation of the lyso-types of the pathogen of rice bacterial leaf blight in the Hokuriku District revealed that lyso-type A bacteria are most prevalent, about 2/3 of the total. Then lyso-type B covering 1/6, and types E and C were small, and type D was not isolated.

14) By using the characteristics of OP₂, such as its wide host range, its long latent period, and its large amount of burst, quantitative tests of various samples is possible.

15) The viability of the pathogen of rice bacterial leaf blight in soil as tested by the phage method of cultured bacteria is 50 days at 3°C during winter under outdoor conditions, five days during summer at 27°C under outdoor conditions, 26 days in a refrigerator at 5°C, and 11 days in a thermostat at 28°C. With the streptomycin resistant test the pathogen would die out in five days at less than 27°C, and in 13 days at 19-22°C. However, further examination of these results is necessary.

16) The dried bacterial ooze of the pathogen of rice bacterial leaf blight secreted and formed on diseased leaves survives both in dry and wet soil until the following April, and pathogenicity was shown toward rice plants.

17) When dried bacterial ooze is preserved in a dry desicator and in a refrigerator at 5°C, it preserves its vitality for two years, and shows pathogenicity toward rice plants.

18) The viability of the pathogen of rice bacterial leaf blight in irrigation water during winter was 15 days in the case of cultured bacteria.

19) The test on the overwintering of the pathogen of rice bacterial leaf blight in cut stems showed that the rate of overwintering and survival of cut stems was 0% and bacteria die by the end of December.

20) The overwintering and viability of bacteria in infected straw is until the following May in the case of straw stored inside, or of inside straws stacked outside; however bacteria die by November in the straws stacked on rice racks, or outside straws left outdoors. The limit of viability of bacteria was 15 days in the case of bacteria in straws abandoned in fields.

21) The overwintering and survival of the pathogen of rice bacterial leaf blight in seeds collected from infected stems lasted until mid-March, but this could be longer depending on the degree of disease in seeds. The position of bacterial latency in seeds seemed to be in the tissues of inner and outer paleas.

22) The observations on the rhizo-spheres of the Leersia oryzoides (Linn.) Sw. during winter in the Hokuriku District showed that the rhizo-spheres are tense and full and maintain a green color until the following spring. New buds grow out in late March.

23) Morphological characteristics, vegetation, and fructification of intermediate host plants of the pathogen of rice bacterial leaf blight such as Leersia oryzoides (Linn.) Sw., Leersia japonica Makino, and Leersia oryzoides (Linn.) Sw. var. japonica (Rets) Honda which are abundant in the Hokuriku District were compared and observed. Through this, differences before young ear formation among these similar plants were clarified.

24) The investigation of the overwintering of the pathogen of rice bacterial leaf blight in the rhizo-spheres of Leersia oryzoides (Linn.) Sw. var. japonica (Rets) Honda showed that bacteria clearly survived for two months from November to the following early January, but no bacteria were detected until late April. And, a large amount of bacteria was detected again in mid-May.

25) The overwintering of bacteria in the rhizo-spheres of Leersia oryzoides (Linn.) Sw. and a few other intermediate host plants was investigated. The results showed the survival of bacteria in Zizania latifolia (Turcz) and Leersia japonica Makino until December, and only a small amount of bacteria perhaps due to contamination was recognized in November in Isachne globosa (Thunb) O. Kuntz.

26) Electron microscopic observations of dried bacteria ooze, which is importantly related to the overwintering of bacteria, showed high bacteria electron density in the drying process of autumn corresponding to long survival.

27) The investigation of fluctuations in the occurrence of rice bacterial leaf blight in nurseries revealed that no occurrence was recognized in seedlings, but the infection of seedlings was discovered in relationship to the source of seedlings.

28) The fluctuation of the pathogen of rice bacterial

leaf blight in seedlings is not clear because the amount of bacteria detected in nurseries is generally small. However it seems that the amount of bacteria seems to increase toward the latter part.

29) The curve depicting the fluctuation of seedlings in paddy fields and the process of occurrence shows two peak periods. The one is the first period of increase in the intermediate tillering stage (the first occurrence period), and the other is the second period of increase after the young ear formation period (period of typhoons). There is the summer stagnation period between these two peaks when the disease progress comes to a standstill and bacterial multiplication is suppressed.

30) The amount of bacteria in the first period of increase and the degree of occurrence are influenced by the amount of infected bacteria in seedlings or in the first period of paddy fields. And the amount of bacteria and the degree of occurrence in the second period of increase are closely related to the amount of bacteria and the degree of occurrence in the first period of increase and the typhoons during the latter half of rice growth.

31) The relationship between the forms of nurseries and the occurrence of the disease in paddy fields is that those seedlings that were planted and grown in warm mixed type nurseries and wet nurseries show early occurrence in paddy fields after transplanting, and with a more intense degree of occurrence.

32) The investigation of the amount of bacteria in seedlings and the inoculation time and frequency in the latter half of nurseries revealed that seedlings with immersion inoculation have more occurrence in proportion to the concentration of bacteria, that those seedlings which are transplanted to paddy fields after a certain multiplication period result in more occurrence and severe occurrence.

33) The reason for the temporary stagnation of bacterial multiplication on rice leaves during the high temperature period in summer was investigated. The result was that bacteria in a glasshouse at an average highest temperature of 35°C with the lowest humidity of 50%, clearly showed suppression of multiplication on rice leaves.

34) Several tests were made on wind dissemination, which plays an important role in the aspect of the secondary infection of rice bacterial leaf blight. They clarified

that bacteria were dispersed as far as 64 m with a maximum wind velocity of 28 m/sec. The distance margin for the occurrence due to the dispersed bacteria was within 4 m. Bacterial dispersion agreed with the direction of the wind. Bacterial dispersion by wind accompanied by rain showed more dispersion.

35) From 1959 to 1960, the following investigation was made in Niigata Prefecture in order to clarify the relationship between the phage of the pathogen of rice bacterial leaf blight in irrigation waters of nurseries and paddy fields, and the occurrence of this disease.

a) Although the number of phages in the irrigation water in the nurseries is generally small, the number detected in the frequently infected year is more than in the year of small occurrence, up to a maximum of 26/ml of phage.

b) When many phages are detected in nurseries, the number of phages is increased in paddy fields even after transplanting and have a trend of more intense occurrence.

c) The number of phages shows a gradual increase in paddy fields. However, the extent and degree of increase vary with the timing of the occurrence of the disease in rice, and its extent. Thus in paddy fields with early occurrence, over 10^3 /ml of phage is detected in the intermediate tillering stage, and over 10^4 /ml in the peak tillering stage in the surface water of paddy fields. Such paddy fields have severe damage from occurrence.

d) In paddy fields where the phage in the surface water during the intermediate tillering stage is over 100/ml, the degree of occurrence is more than "medium" and when it is less than 50/ml, the degree of occurrence tends to be "small."

36) The following was clarified through the investigation made in 1959 in Niigata Prefecture on the relationship between the number of phages in irrigation channels and occurrence.

a) The number of phages in irrigation channels shows a gradual increase beginning in the early period of paddy fields, and when the number reaches 1,000-2,000, the occurrence of the disease is observable in nearby paddy fields.

b) When the number of phages in irrigation

channels is less than 50 per 1 ml, the occurrence of the disease is observable in Leornia oryzoides (Linn.) Sw. When it is 100-200/ml, this corresponds to 10-14 days prior to the discovery of the first occurrence in rice.

c) The number of phages in paddy fields where the first occurrence in rice is observable, is over 10^4 per 1 ml.

37) The following was clarified by the investigation, for a four year period, from 1958 to 1961, with the Hokuriku District Agricultural Experimental Station and its vicinity as the objective in order to clarify the relationship between the number of phages in rivers or main water channels, and the occurrence of the disease in the area.

a) The timing of the detection of phages in rivers and the main water channel corresponds to the timing of the occurrence of the disease in the area.

b) When the number of phages in rivers and the main water channels is over 100, the occurrence of the disease in rice is observable in nearby areas.

c) The relationship between phage fluctuation in rivers and water channels and the process of occurrence is a close parallel one, and a trend corresponding to the fluctuation of all the bacteria is observable.

38) Because of the relationship between the number of phages in nurseries, paddy fields, irrigation channels, water channels, and river irrigation channels, and the occurrence of the disease, by measuring the phages in irrigation water at the points set up for measurement along drainage routes of infected areas, and the lower reaches of water channels and rivers where such water flows into, data for forecasting the timing and degree of the occurrence of this disease in the region or locality concerned is possible.

39) Tests were made on several problematic points to be looked for in measuring the phages in irrigation water. Thus the points to be taken with caution were pointed out in selecting the indicator strains for the detection of phages, and in collecting irrigation water samples.

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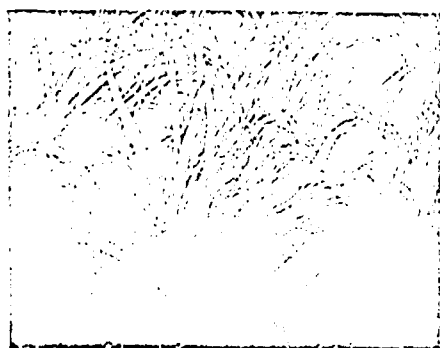
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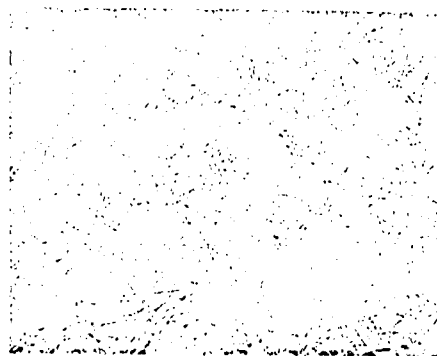
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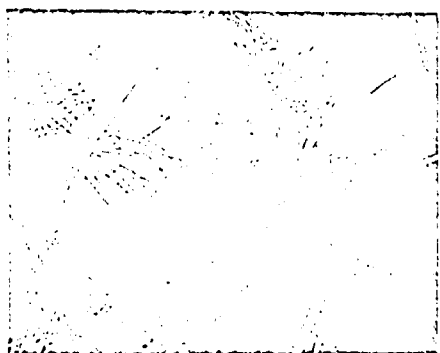
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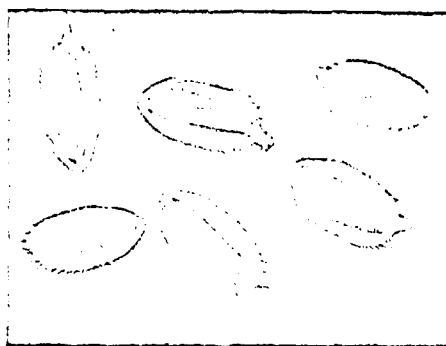
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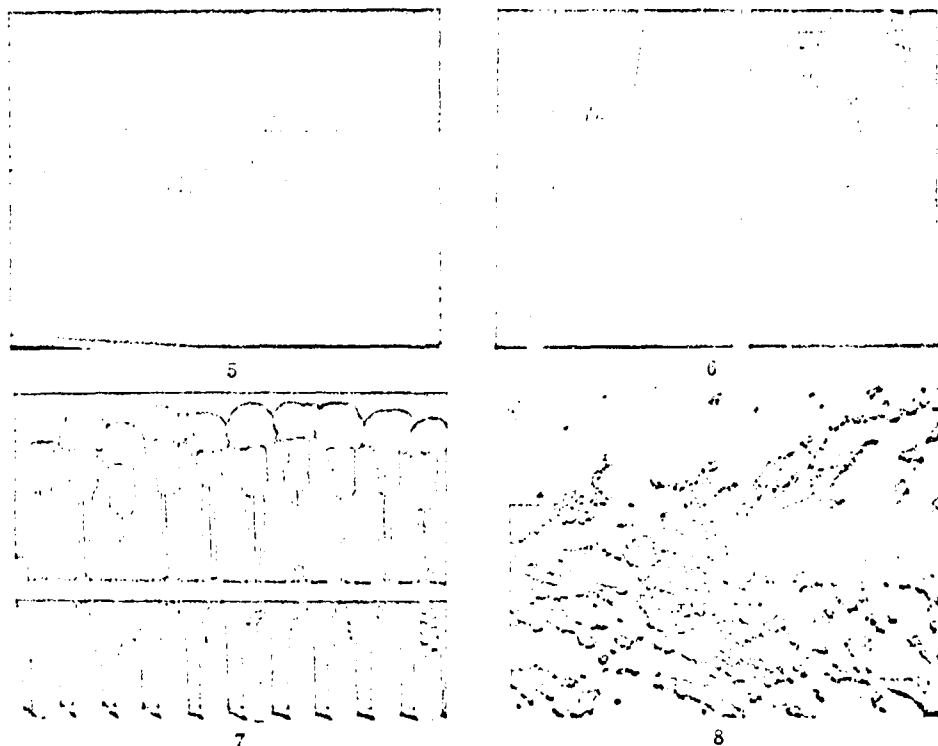
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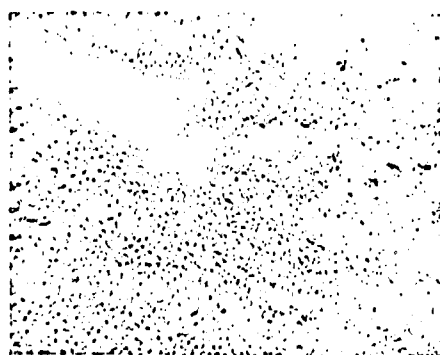
1. Occurrence of rice bacterial leaf blight in Hokuriku Agricultural Experimental Station, Takada, Niigata Prefecture, name of rice variety: Jukkoku.
2. "Zurikomi", a kind of wilting phenomenon caused by rice bacterial leaf blight, place: Shimonota, Takada, Niigata Prefecture, name of rice variety: Sanin 52, 1959.
3. Ditto, place: Aburaden, Takada, Niigata Prefecture, name of rice variety: Koshisakae, 1961.
4. Disease symptom on un-hulled rice, name of rice variety: Kinmaze.

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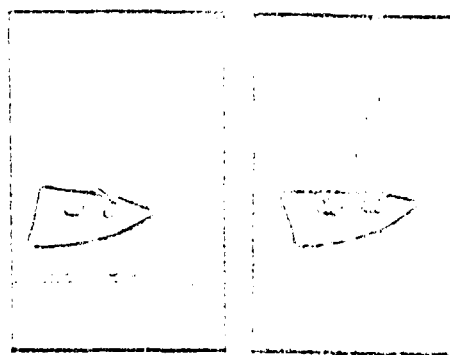


5. Bacterial ooze of Xanthomonas oryzae (Uyeda et Ishiyama) Dowson on rice leaf, name of rice variety: Minmaze.
6. Bacterial secretion from affected rice leaves in water.
7. PPSA (potato pepton sucrose agar) medium culture of X. oryzae, incubated at 27°C 5 days.
8. Capsule-like membrane (it may be so-called slime layer), staining by Hiss's method.

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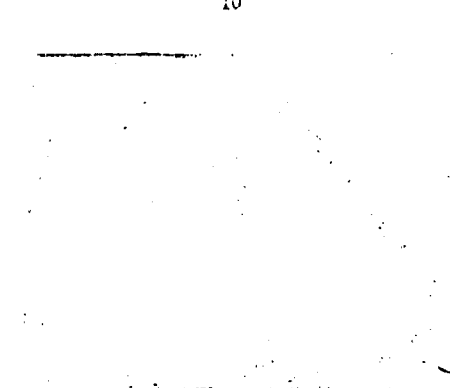
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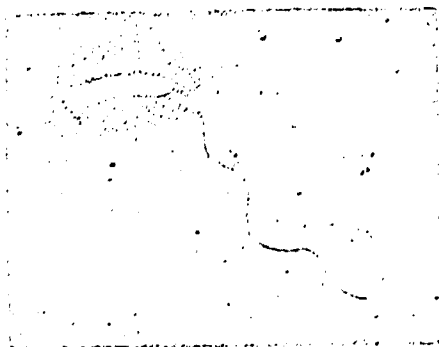
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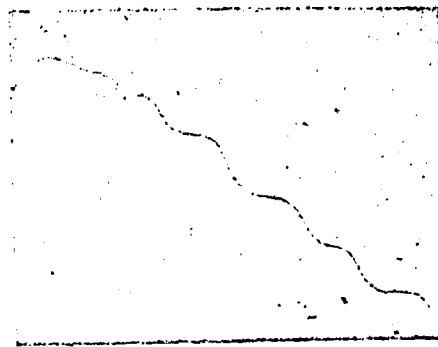
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9. Capsule-like membrane (it may be so-called slime layer), staining by Novelli's method.
10. Showing the method of mesh mounting of each electron-microscope observation; right: in the case of affected leaf, left: in the case of capillary pipette using.
11. Electron micrograph of dispersion of bacterial cells grown in host tissue, undispersal and heavy slimy.
12. Ditto, onlargement x 16,000.

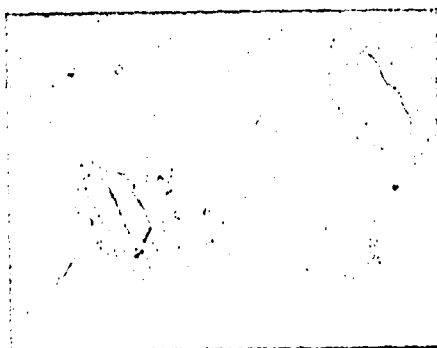
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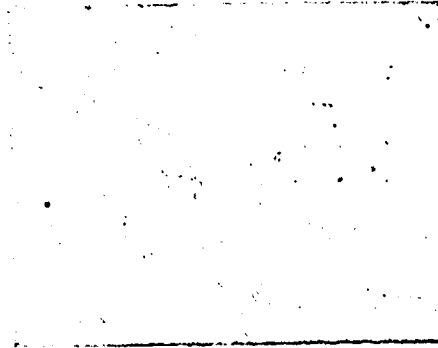
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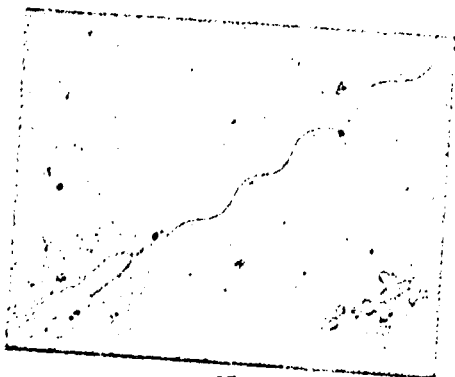
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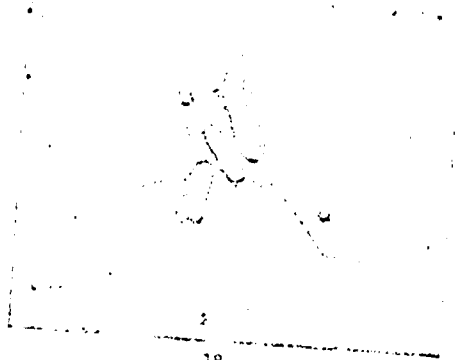
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13. Electron micrograph of *X. amurensis*, just before cell division, lyso-type A, name of isolate: Shinjo, x 16,000.
14. Ditto, lyso-type A', length of flagella measured in 6.75μ , isolate No.: H-5921.
15. Ditto, lyso-type B, capsule-like membrane is very clear, name of isolate: Beniya.
16. Ditto, lyso-type C, isolate No.: H-5629.

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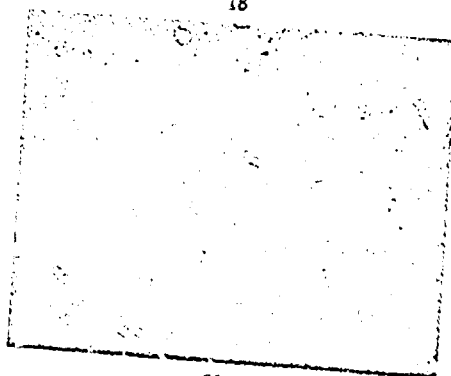
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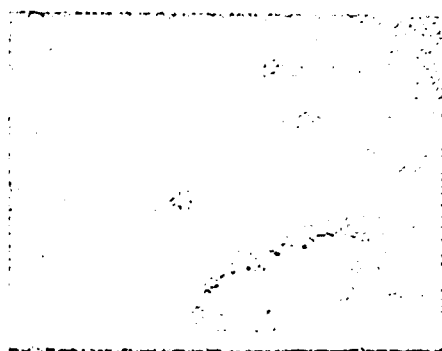
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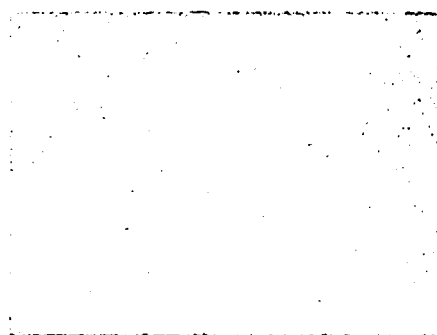
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17. Electron micrograph of *X. oryzae*, just before cell division, lyso-type D, isolate No.: H-5925.
18. Ditto, lyso-type E, isolate No.: H-5913.
19. Electron micrograph of *X. oryzae* bacteriophage particles named OP₂, x 20,000.
20. Ditto, OP_{2m} phage particles.

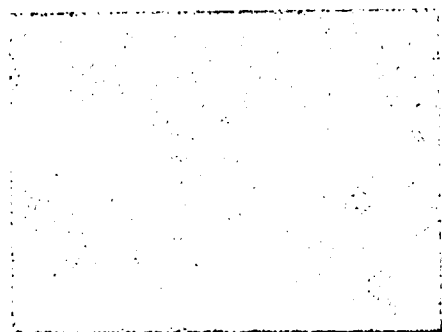
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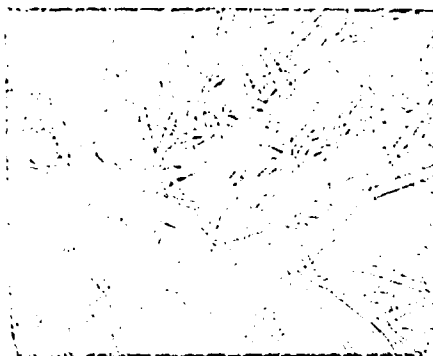
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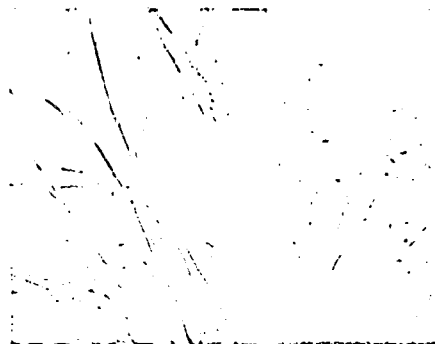
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21. Electron micrograph of *X. gryzax* bacterio-phage particle named OP₁ phage particles.
22. Ditto, OP_{1h} phage particles.
23. Ditto, OP_{1h2} phage particles.
24. Plaques produced by *X. gryzax* phage strains after 26 hours on P2SA medium plate cultures, 1: plaques of OP₁ phage for X. g., Shingo, lyso-type A, 2: plaques of OP₂ phage for X. g., H-5913, lyso-type B, 3: plaques of OP_{2m} phage for X. g., H-5921, lyso-type A'.

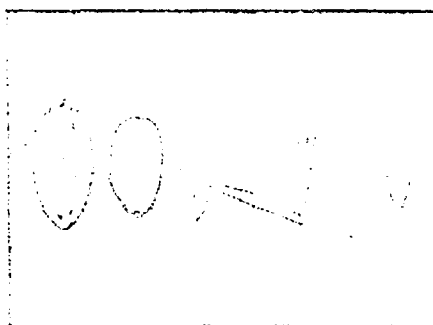
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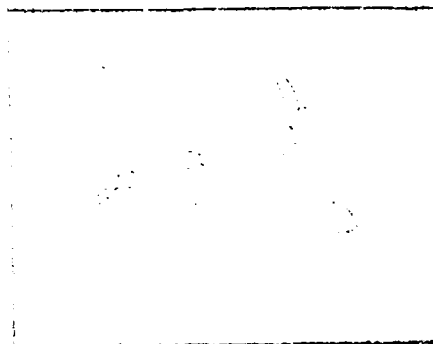
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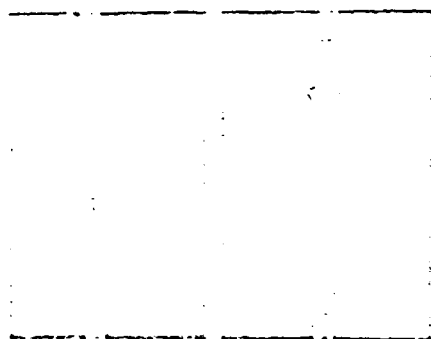
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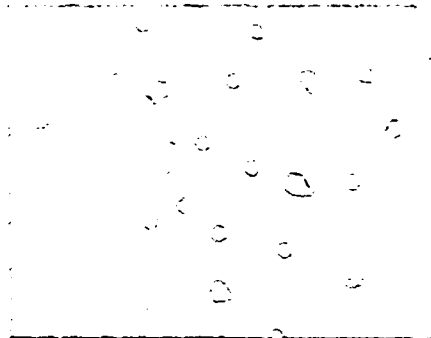
28

25. Occurrence of bacterial leaf blight on intermediate host plant, Leersia oryzoides (Linn.) Sw.
26. Diseased leaves of bacterial leaf blight by needle inoculation on Foxtail, Setaria viridis Beauv.
27. Fructification of seed in intermediate host plant, left: rice, center: L. oryzoides, right: L.o. var. japonica (Honda) Chi.
28. Germination test of L.o. var. japonica seed by rind cutting, lower right: non-treatment seed.

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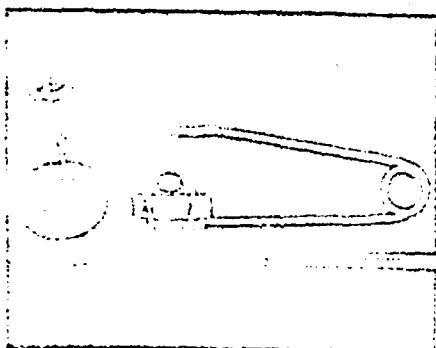
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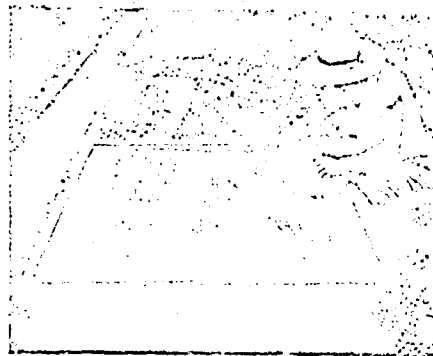
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29. Difference in morphological characteristics of the similar intermediate host woods of Graminaceae, left: L.c., right: L. japonica Makino.
30. Ditto, left: L. oryzoides (Linn.) Sw. var. japonica (Honda) Ohi, right: L. oryzoides (Linn.) Sw.
31. Dry bacterial ooze.
32. Electron micrograph of X.o. bacteria cells grown in host tissue at harvest time. High-electro-dense cells are observed.

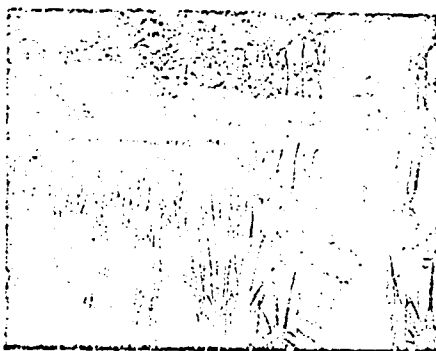
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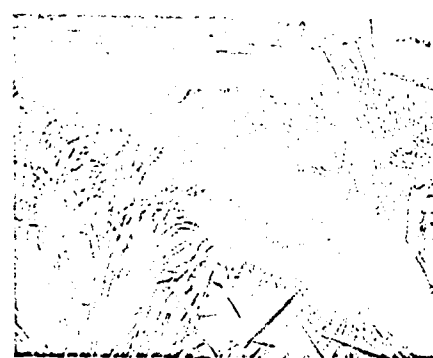
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36

- 33. Needle inoculation pancher, the Hokuriku Agr. Exp. Sta. type.
- 34. Showing the sink inoculation method of seedlings.
- 35. Several intermediate host plants growing in and around reservoir in occurrence area of this disease, place: Aono-jumonji, Naoetsu, Niigata Prefecture, 1958.
- 36. Ill-drained paddy field widely extending in occurrence area of this disease, in Hokuriku District.

- END -

- 302 -